Attorney Docket No. 5470-401 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Stafford et al.
Serial No.: 10/573,131
Filed: April 18, 2006
Confirmation: 4529
Group Art Unit: 1634
Examiner: Jehanne S. Sitton

For: **METHODS AND COMPOSITIONS FOR THE CORRELATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE VITAMIN K EPOXIDE REDUCTASE GENE AND WARFARIN DOSAGE**

Mail Stop Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Date: May 29, 2009

COMMUNICATION TO SUBMIT DECLARATION UNDER 37 C.F.R. § 1.131

Sir:

On April 28, 2009, Applicants filed a response to the July 28, 2008 non-final Office Action issued for the above-referenced patent application. In that response, Applicants stated that a Declaration under 37 C.F.R. § 1.131 would be submitted to address the rejections of the pending claims based on the Oldenburg et al. and Rost et al. references. Applicants now submit the Declaration under 37 C.F.R. § 1.131 described in the April 28, 2009 response. Applicants respectfully request entry of this Declaration into the present application and consideration with the response filed April 28, 2009.

No fee is believed due with this communication. However, the Commissioner is authorized to charge any deficiency or credit any overpayment to Deposit Account No. 50-0220.

Respectfully submitted,

Mary L. Miller
Registration No. 39,303

Customer No. 20792
Myers Bigel Sibley & Sajovec, P.A.
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CERTIFICATION OF ELECTRONIC TRANSMISSION
I hereby certify that this correspondence is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4) to the U.S. Patent and Trademark Office on May 29, 2009.

Claire Wimberly

Attorney Docket No. 5470.401 PATENT

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Declaration of Darrel W. Stafford and Tao Li under 37 C.F.R. § 1.131

We, Darrel W. Stafford and Tao Li, hereby declare and say as follows:

1. We are the named inventors on U.S. Patent Application Serial No. 10/573,131 ("the '131 application").

2. Prior to October 14, 2003, we conceived of the invention as recited in the pending claims. In addition, we exercised due diligence from prior to October 14, 2003 until reduction to practice of the invention.

3. In support of the statements of paragraph 2 above, attached hereto are **Tabs 1-34,** in which documentation is provided of the conception and reduction to practice of the present invention.

Tab 1 is a copy of an Application for Approval of Research Involving Human Subjects as submitted to the University of North Carolina. While the date of the Application has been redacted, the date is prior to October 14, 2003. This document demonstrates the conception of:

(1) the method of identifying a human subject having an increased sensitivity to warfarin, comprising detecting in the subject the presence of an allele of a single nucleotide polymorphism in the VKOR gene, wherein the allele of the single nucleotide polymorphism is correlated with increased sensitivity to warfarin;

2) the method of identifying a human subject having increased sensitivity to warfarin, comprising: a) correlating the presence of an allele of a single nucleotide polymorphism in the VKOR gene with increased sensitivity to warfarin; and b) detecting the allele of the single nucleotide polymorphism of step (a) in the subject, thereby identifying a subject having increased sensitivity to warfarin; and

3) the method of amplifying a segment of a VKOR genomic nucleotide sequence, wherein said segment is in a noncoding region of the nucleotide sequence, comprising: a)

choosing a first oligonucleotide primer from the nucleotide sequence of SEQ ID NO:8; b) choosing a second oligonucleotide primer from the nucleotide sequence of SEQ ID NO:8; c) adding said first primer and said second primer to a nucleic acid sample; and d) amplifying a segment of the VKOR genomic nucleotide sequence defined by the first primer and the second primer, wherein said segment is in a noncoding region of the nucleotide sequence, further wherein the amplified segment of step (d) comprises a single nucleotide polymorphism and/or an allele of a single nucleotide polymorphism that is correlated with increased sensitivity to warfarin, and/or wherein the nucleic acid sample is from a subject for whom identification of an increase or decrease in warfarin sensitivity is desired.
(See, in particular, page 4.)

Tab 2 is a copy of an electronic mail (e-mail) to Dr. Stafford discussing the use of the University of North Carolina (UNC) coagulation lab for collecting samples to test for VKOR polymorphisms; October 24, 2003.

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4. International Application No. PCT/US2004/031481 was filed on September 23, 2004 and published on April 7, 2005 as PCT Publication Number WO2005/030039.

5. In summary, our statements herein and the documents concurrently submitted show conception prior to October 14, 2003 and diligence prior to that date until a reduction to practice of the claimed invention.

6. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Darrel W. Stafford

Date

Tao Li

05/27/2009
Date

Enclosures: Tabs 1-34

Attorney Docket No. 5470.401 PATENT

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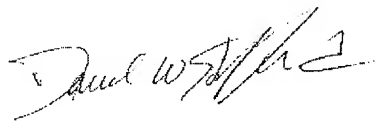


Darrel W. Stafford

05/28/09
Date

Tao Li

Date

Enclosures: Tabs 1-34

TAB 1

University of North Carolina, Chapel Hill
Committee on the Protection of the Rights of Human Subjects (Medical IRB)

APPLICATION FOR APPROVAL OF RESEARCH INVOLVING HUMAN SUBJECTS

DATE: IRB STUDY NUMBER (leave blank if new submission): _____

TITLE OF STUDY: Vitamin K epoxide reductase (VKOR) gene polymorphisms and Coumadin response

NAME AND DEGREE(S) OF PRINCIPAL INVESTIGATOR: James P. Evans, MD, PhD **DEPT:** Genetics
PID NUMBER OF PRINCIPAL INVESTIGATOR: 703458364
MAILING ADDRESS:CB# 7110, UNC Chapel Hill, 27599-7110

PHONE: 966-2276 **FAX:** 966-6735 **PAGER:** 216-0373
E-MAIL: jpevans@med.unc.edu

NAMES AND DEGREE(S) OF CO-INVESTIGATORS: Darrel Stafford, MD

NAME AND PHONE NUMBER OF RESEARCH COORDINATOR, IF APPLICABLE: Lisa Susswein, MS, 919-843-3158, susswein@med.unc.edu

NAME OF FUNDING SOURCE:

I. Agreements

Principal Investigator:
I certify that each of the above-named co-investigators has accepted his/her role in this study. I agree to a continuing exchange of information with the Committee on the Protection of the Rights of Human Subjects (IRB). I agree to obtain IRB approval before making any changes or additions to the project. I will provide progress reports at least annually, or as requested. I agree to report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. A copy of the consent form will be given to each subject and the signed original will be retained in my files. If the study involves treatment of UNC Hospitals patients, a copy of the consent form will be placed in each subject's medical record.

_____ _____
Signature of Principal Investigator Date

_____ _____
Signature of Faculty Advisor if P.I. trainee or Non-Faculty Date

Department Chair of P.I. (or Vice-Chair if Chair is investigator or otherwise unable to review):
I have reviewed this research study. I believe the research is sound, that the study design and methods are adequate to achieve the study goals, and that there are appropriate resources (financial and otherwise) available to the investigator. I support it, and hereby submit it for further review.

_____ _____ _____
Signature of Department Chair Department Date

II. Summary Checklist

ARE THE FOLLOWING INVOLVED?	YES	NO
Surveys, questionnaires or interviews	☐	☒
Existing Patient Records and/or Specimens *If research is limited to study of existing medical records and /or samples, Submit **Short Form** instead of this application.*	☒	☐
Investigational Drug(s) (provide IND # _____) Approved drugs for "non-FDA-approved" conditions *All studies involving drugs or supplements must use the Investigational Drug Service (IDS).*	☐ ☐	☒ ☐
Investigational devices, instruments, machines, software (provide IDE #_____)	☐	☒
Placebo(s)	☐	☒
Genetic studies on subjects' specimens	☒	☐
Storage of subjects' specimens for future, as-yet-undesignated research *If "yes", see **Instructions for Submitting IRB Applications for Research that Includes the Storage of Human Biologic Specimens.***	☒	☐
Fetal tissue	☐	☒
Videotaping, audiotaping, filming of subjects	☐	☒
Non-patient volunteers	☐	☒
Patients as subjects	☒	☐
Minors (less that 18 years old) *If "yes", indicate:* **Age range to years**	☐	☒
Do you intend to target your enrollment at: -Students or staff as subjects?	☐	☒
-Non-English-speaking subjects?	☐	☒
-Decisionally impaired or mentally incompetent subjects?	☐	☒
-Prisoners, parolees and other convicted offenders as subjects?	☐	☒
-Pregnant subjects?	☐	☒
Will HIV tests be performed?	☐	☒
Will subjects be studied at off-campus sites?	☐	☒
Is this a multicenter study? *If "yes", is UNC-CH the sponsor or coordinating center?*	☐	☒
Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise *If "yes", approval by the Radiation Safety Committee is required.*	☐	☒
Recombinant DNA or gene transfer to human subjects *If "yes", approval by the Biologic Safety Committee is required.*	☐	☒
Is this an oncology study? *If "yes", submit this application directly to the Oncology Protocol Review Committee.*	☐	☒
Will subjects be studied in the General Clinical Research Center? *If "yes", obtain GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	☐	☒

III. Required Education in Human Subjects Protection

UNC policy requires that all persons engaged in research involving human subjects must complete training in ethical conduct of research and protection of subjects. This applies to all research, regardless of funding source. For further information, including what options are acceptable in fulfillment of these requirements, see http://www.med.unc.edu/irb/Education2.htm.

Individuals who have completed training should have been entered into the Human Subjects Training Database maintained by the Office of the Vice Chancellor for Research and Economic Development. To print documentation, visit http://cfx.research.unc.edu/IRBcert/search.cfm and enter the names of each individual involved with this research project. Names not returned by the database are not recognized as having satisfied the education requirement. For questions regarding the database, please contact (Judy Christman, CB 4100; fax 962-4100).

WITH THIS APPLICATION, please submit the printout from the Human Subjects Training Database, verifying that each individual involved in the research (including faculty, staff, students and outside collaborators, if responsible to this IRB) has satisfied the education requirements.

IV. Potential Conflict of Interest

The following questions apply to any investigators or study staff involved with industry-sponsored research, and/or their immediate family members (spouse, dependent children, others). Within the past 12 months or the next 12 months, have you or will you:

Receive any form of personal compensation from the Sponsor, including salary, consulting fees, honoraria, royalties, equipment, etc.?	☐ YES	☐ NO
If so, does or will that compensation exceed $10,000?	☐ YES	☐ NO
Have an ownership interest of any nature in the Sponsor or product under study, including equity, stock options, etc.?	☐ YES	☐ NO
If so, does or will that interest exceed $10,000 in value?	☐ YES	☐ NO
If so, does that interest represent more than 5% ownership in the Sponsor?	☐ YES	☐ NO
Hold any position with the Sponsor, including officer, director, trustee, consultant, member of advisory board, etc.?	☐ YES	☐ NO
Have an intellectual property interest on any technology or invention used in this study, including patent rights, copyright, etc.?	☐ YES	☐ NO
Have a conflict of interest disclosed through the University's annual evaluation policy that relates to this research study?	☐ YES	☐ NO

If the answer is "YES" to any of the questions above, please include an explanation with this application. As with any changes to the research itself, relationships or interests that develop later should be brought to the IRB's attention for further consideration.

V. Description of Proposed Research Activity

Entire application should not usually exceed 5 single-spaced pages using a 12-point font.

1. **Purpose and Rationale:** Provide a brief summary of the background information, state the research question(s), and tell why the study is needed. Avoid an extensive literature review in this document, unless there is no supporting master protocol.

Warfarin is widely prescribed in order to reduce thrombo-embolic complications in patients at risk for such disorders. However, it is a notoriously difficult medication to use because of its narrow therapeutic index and extreme variability regarding dosing and efficacy in those for whom it is prescribed. Patients have widely varying dosing requirements with some individuals being very sensitive to small amounts of the drug, while others require large doses to achieve an appropriate anti-coagulant effect. Moreover, in a given individual the appropriate dose often varies unpredictably, necessitating frequent monitoring of patients who take this medication. The molecular basis for this variability is unknown at present.

Recently the gene which encodes the molecular target of warfarin, vitamin K epoxide reductase (VKOR), was recently cloned by one of us (manuscript submitted). It is hypothesized that genetic polymoprphisms in the VKOR gene exist which are responsible for the variability in dosing requirements among patients. Knowledge of an individual's genotype would be valuable as a way to rationally determine dosage requirements for patients who need this medication, and for the identification of patients who are likely to need either increased or decreased monitoring while on therapy.

The purpose of the proposed study is to:

1. Sequence the coding regions of the VKOR gene from patients in the coagulation clinic in order to search for polymorphisms in this gene.
2. Analyze any such polymorphisms for correlation between their presence and dosing requirements in patients taking warfarin.

2. **Subjects:** Specify number, age, gender, ethnicity, and whether healthy volunteers or patients. If patients, specify the disease or condition and indicate how potential subjects will be identified. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided. NIH applications require that women, minorities, and children be included or that their exclusion be justified. If children are involved, refer to "Children as Research Subjects".

Eligible subjects will be patients who have been taking warfarin for at least six months and who have been followed in the UNC Coagulation clinic at the Ambulatory Care Clinic. We propose to enroll 150 consecutive patients of 18 years or older. All ethnicities and racial backgrounds are eligible. All individuals will be patients who are taking warfarin for its anti-thromboembolic properties. This generally will include patients who are at increased risk of thromboembolism due to the presence of chronic or paroxysmal atrial fibrillation, mechanical heart valves, or a hypercoagulable state.

Preganant women are not given warfarin due to its teratogenic effects and thus will not be represented in the study. Likewise, children are not generally on this medication and will be excluded.

3. **Inclusion/Exclusion criteria:** List required characteristics of potential subjects, and those that preclude enrollment.

Subjects will be 18 years or older and will have been taking warfarin and have been followed in the Coagulation Clinic for at least six months. Consecutive subjects will be ascertained as they come to clinic for their pre-arranged appointment for monitoring of their coagulation status. Pregant women and children are typically not treated with this agent and thus will not be represented in the study.

4. **Full description of the study design, methods and procedures:** Include the type of experimental design; study procedures; sequential description of what will be asked of/done to subjects; assignment of subjects to various arms of the study if applicable; doses, frequency and route of administration of medication and other treatment if applicable; kinds of data to be collected; primary outcome measurements; and follow-up procedures. If the study involves treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls. This section (4) should generally not exceed 2 single-spaced pages using 12-point type.

Following informed consent, a total of 14 cc of blood will be drawn by standard procedures into two ACD containing tubes. Upon receipt of the blood by Dr. Stafford's laboratory, DNA will be extracted and the coding regions of the VKOR gene will be sequenced.

The sequence of each individual's VKOR gene will be analyzed for the presence of polymorphisms (genetic differences among individuals in the population).

If polymorphisms are identified, statistical correlations will be sought between such genetic variations and the warfarin dosing requirements and past bleeding complications of the patients.

Dosing requirements and coagulation parameters of the patients will be obtained, with the patient's informed consent, by analysis of the database in the coagulation clinic which monitors patient's warfarin doses, their degree of anticoagulation, and warfarin related complications. Access to the UNC clinical information system will be used to evaluate the relationship between genotype and likelihood of clinical complications from warfarin use.

Primary outcomes will consist of:

1. Whether polymorphisms exist in the VKOR gene in the population of individuals followed by the UNC coagulation clinc.
2. Whether such polymorphisms are related to variability in patient's dosage requirements.
3. Whether such polymorphisms are related to the incidence of clinical complications secondary to warfarin treatment.

Information resulting from this study would be preliminary and thus will not lead directly to any changes in the clinical care of patients.

5. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable**: Include the number of required visits and approximate duration of each visit.

Participation will involve obtaining informed consent and a blood sample during a routine visit that the patient has already scheduled for purposes of warfarin monitoring in the UNC Coagulation Clinic.
We estimate that it will take approximately one month to collect samples from the proposed 150 patients. No follow-up visits will be necessary.

6. **Where will the subjects be studied?** If off UNC-CH campus, list locations.

Subjects will be approached and consented in the UNC Coagulation Clinic in the Ambulatory Care Center. DNA will be extracted from the blood samples and analyzed in the laboratory of Dr. Darrell Stafford at UNC in 442 Wilson Hall.

7. **Full description of risks and measures to minimize risks:** Include risk of psychosocial harm (e.g. emotional distress, embarrassment, breach of confidentiality, etc.) economic harm (e.g. loss of insurability) and legal jeopardy

(e.g. disclosure of illegal activity) as well as known side effects of study medication, if applicable, and risk of pain and physical injury.

Risk is minimal. A small risk of hematoma or bruising is inherent in the blood draw process. Samples will be assigned a tracking number to maintain patient confidentiality. The principle investigators will be able to link clinical information to the VKOR genotype in order to correlate genotype with warfarin dosage requirements. Such information will be kept confidential, and would not have implications for insurability, legal jeopardy, or other potential harm.

8. **Benefits to subjects and/or society:** The possibility of benefit to society should be clearly distinguished from the possibility of benefit to the individual subject, if any. If there is no direct benefit to the individual subject, say so. Do not list monetary payment as a benefit.

There will be no direct medical benefits to subjects. From a societal standpoint, the discovery of polymorphisms which influence warfarin dosage could lead to considerable improvements in the safe use of this widely used agent.

9. **Inducements for participation:** If monetary, specify the amount and how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it.

There will be no inducements for participation.

10. **Costs to be borne by subjects:** Include clinic fees, diagnostic and laboratory studies, drugs, devices, transportation, all professional fees, etc. If there are no costs to subjects, indicate this.

Subjects will not have any additional costs related to this study.

11. **Statistical analysis:** If this is a single-center study, provide evidence that the sample size is sufficient to achieve the study aims and tell how the data will be analyzed. If a multicenter trial, indicate where and by whom statistical analysis will be performed.

Genetic polymorphisms are common in the general population, but no predictions can be made regarding whether such variability exists in the VKOR gene. If such polymorphisms are found, a Chi-square test will be applied to determine whether these variants are correlated with warfarin dosage requirements.

12. **Methods of recruiting:** Tell how prospective subjects are contacted. If they are UNC Hospital patients, initial contact should be made by their treating physician, or by someone whom the patients know to have legitimate access to their medical records (for example, a clinical director). This may be accomplished by means of a letter from that individual to prospective subjects, requesting the patient's permission to be contacted by the investigator.

Potential subjects will be identified through the UNC Coagulation Clinic and will be first approached by the Coagulation Clinic supervisor (B.B.) who has an ongoing repore with all of the patients and who regularly consults each patient's medical records in the course of her duties. They will be approached in the clinic when they are present for their routine monitoring, which typically occurs every month or more frequently.

13. **How will informed consent be obtained?** Describe the process. When the consent of a legally authorized representative is substituted for consent of the adult subject, explain why this is necessary. If non-English-speaking subjects will be enrolled, a consent form should be prepared in their foreign language. Someone who is fluent in the subjects' language must be available to interpret.

Informed consent will be obtained by a trained genetic counselor or research assistant. Patients not fluent in English will not be eligible for the study due to the need for consent and the limited availability of translators.

Submit the following to: IRB Office, Building 52, CB# 7097, UNC-CH; GCRC studies to: GCRC, University of NC, Room 3005, APCF, CB #7600, Chapel Hill, NC 27599-7600

Original and 2 copies of this completed, signed application;
3 copies of each consent and assent form;

If applicable, submit also:
- **3 copies of the Master Protocol; represented by the full application if NIH/DHHS grant**
- **1 copy of the Investigator's Brochure;**
- **3 copies of questionnaires or survey instruments;**
- **3 copies of recruitment materials (letters, ads, posters, TV or radio scripts).**

Please do not send double sided copies.

For addition information consult the IRB website at http://www.med.unc.edu/irb/, email us at irb_questions@med.unc.edu, or call the IRB Office at 966-1344.

TAB 2

Darrel Stafford

From: Evans, Jim [jpevans@med.unc.edu]
Sent: Friday, October 24, 2003 10:11 AM
To: 1lisa Susswein (susswein@med.unc.edu)
Cc: Skrzynia, Cecile; Darrel Stafford; Evans, Jim
Subject: coag study

Hey guys,

Cecile and I just met with rob malone in the coag clinic. They really have virtually everthing we need in their database; it's great. Average inr, average coumadin dose, variance in dose, comorbidities, other meds they're on, etc.

They see 10-12 patients each half-day. They have plenty of room so that is not a problem. I think that in just a couple of days we could get a first installment of at least 20 blood samples and see if polymorphisms are present. If so, then we can collect more.

Here is our big challenge: to do this efficiently we need to avoid sending folks down to get their blood drawn in the lab. Our consent rate will plummet if they have to do that. Also, if we have to send folks to the lab we will need to pay the lab somehow for drawing the blood. I'm not sure how much that is, but my guess is that even for 20 patients we're talking anywhere from $250 to $1500.

SO, we need to beg, borrow, or steal someone with phlebotomy training that can hang at the coag clinic to consent people and suck their blood. Lisa, any thoughts? Do you think we could get lisa carey to lend us a phlebotomist for a couple of days?

Here are some other design thoughts for analysis if we see polymorphisms:

1. the important measure would be the ratio of average warfarin dose to average inr. This would correct for the fact that we're happy with some folks sitting at an inr of 1.9 and others sitting with an inr of 2.1. we need to correct for our clinical lack of rigor in dosage acceptance.

2. we need to look at other drugs they are on. Perhaps polymorphisms in vkor are operative especially in the context of other drugs that influence metabolism

3. we should take into account smoking vs. non-smoking (that info is in the database).

Darrel, the IRB application has been submitted. I hope we'll have approval in the next (?Lisa, what do you think the time frame here is?) 2-3 weeks and could start collecting blood.

I think we can figure out the phlebotomy issue. This could be really cool if we do see any polymorphisms.

Later,

Jim

TAB 3

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Mon, 17 Nov 2003 13:31:14 -0500 (Eastern Standard Time)
Subject: VKOR SNP project
To: Lisa_Susswein@med.unc.edu ("Susswein, Lisa")

Hi! Lisa,

How is everything going regarding to drawing blood from patients? Weeks ago I have tested the conditions about extracting genomic DNA from blood, PCR and sequencing. I am quite ready for this project.

Have a nice day!

Tao

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Wed, 3 Dec 2003 15:22:28 -0500 (Eastern Standard Time)
Subject: Blood drawing
To: jpevans@med.unc.edu ("Evans, Jim")
Cc: susswein@med.unc.edu ("llisa Susswein (susswein@med.unc.edu)")

Hi! Dr. Evans,

I will be out of town from tomorrow to Dec 9. Probably we can start the first round of blood drawing after I come back. Dr. Stafford will be also out of town till Dec 15.

Have a nice day!

Tao

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Wed, 10 Dec 2003 16:15:58 -0500 (Eastern Standard Time)
Subject: Re: Coumadin study
To: Lisa_Susswein@med.unc.edu ("Susswein, Lisa"),
    Lisa_Gatti@med.unc.edu ("Gatti, Lisa"),
    Lynda_sawyer@med.unc.edu ("'Lynda_sawyer@med.unc.edu'"),
    jpevans@med.unc.edu ("Evans, Jim"),
    taoli@email.unc.edu ("Tao Li (taoli@email.unc.edu)"),
    rcnp@med.unc.edu ("Skrzynia, Cecile")

Hi! Lisa,

Sorry about my late response. I just came back from San Diego. It's great that we can start it soon. Please tell me where to meet on Thursday morning. Thanks!

Tao

-- Begin original message --

> From: "Susswein, Lisa" <Lisa_Susswein@med.unc.edu>
> Date: Tue, 9 Dec 2003 10:34:14 -0500
> Subject: Coumadin study
> To: "Gatti, Lisa" <Lisa_Gatti@med.unc.edu>,
>   "'Lynda_sawyer@med.unc.edu'"
>   <Lynda_sawyer@med.unc.edu>,
>   "Evans, Jim" <jpevans@med.unc.edu>,
>   "Tao Li (taoli@email.unc.edu)" <taoli@email.unc.edu>,
>   "Skrzynia, Cecile"
>   <rcnp@med.unc.edu>
>
>
> ------_=_NextPart_001_01C3BE69.E6A432F0
> Content-Type: text/plain
>
> We have a start date! Rob has asked that we wait until Monday 12/15 to
> start recruiting patients. However, we will go over on Thursday morning at
> 8:30 for a brief planning meeting/orientation. Lynda or Jim - can you tell
> us where to go?
>
>
>
> Thanks!
>
> Lisa
>
>
>
>
>
> _____
>
> Lisa Susswein, MS, CGC
>
> Genetic Counselor
>
> Cancer Genetics Network
>
> UNC Chapel Hill, CB#7295
>
> Chapel Hill, NC 27599
>
> 919-843-3158 phone
>
> 919-843-7240 fax
>
> sussweinmed.unc.edu
>
>
>
>
> ------_=_NextPart_001_01C3BE69.E6A432F0
> Content-Type: text/html
>
> <html>

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Thu, 11 Dec 2003 13:24:36 -0500 (Eastern Standard Time)
Subject: Re: VKOR
To: Lisa_Susswein@med.unc.edu ("Susswein, Lisa")

Hi! Lisa,

Thank you for setting up the blood drawing! I don't mind picking up the blood sample every day. It's also fine that somebody can deliver them to me. --- Either way works. 5pm-5:30pm of picking-up time is OK for me, but I prefer pick up them as early as possible in the day because it costs 1 hour + 0.5 hour + 2 hours + 1 hour for extracting genomic DNA, setting up PCR, PCR reaction, PCR product purification and mix the sequence sample. I'll try to send samples to sequencing in the next day morning. Hopefully we can deal a batch of samples before sequencing facilities' Christmas vacation. In case you can't find me by calling my lab number 962-2267, please call my cell phone number 360-8663.

Have a nice day!

Tao

-- Begin original message --

> From: "Susswein, Lisa" <Lisa_Susswein@med.unc.edu>
> Date: Thu, 11 Dec 2003 11:40:28 -0500
> Subject: VKOR
> To: "Tao Li (taoli@email.unc.edu)" <taoli@email.unc.edu>
>
>
> ------_=_NextPart_001_01C3C005.7BD238D0
> Content-Type: text/plain
>
> Hi Tao,
>
>
>
> We met with the people in the coag lab this morning. We are all set to
> start drawing blood next week, and will be there in the clinic every day but
> Thursday. Hopefully we will get enough people to do a first run to look for
> polymorphisms. The question now is about the blood. Would you mind coming
> to Lineberger to pick up the blood at the end of the day? One of my
> coworkers parks in the bell tower lot, so on the days when she is here, then
> she would be able to deliver the blood to you. However, she is not always
> here at the end of the day. We can work that out on a day-by-day basis.
> Would it be too late for you to pick up the blood at about 5pm or 5:30? We
> could call you when we get out of the coag clinic.
>
>
>
> Let me know what you think.
>
>
>
> Thanks,
>
> Lisa
>

TAB 4

12/15/2003

3:00pm pick patient's blood samples from

Lisa 843-5942
216-2050 (pager)

#001
#002
#003
} 12/15/03, 03-MED-603
5mL x 2 for each

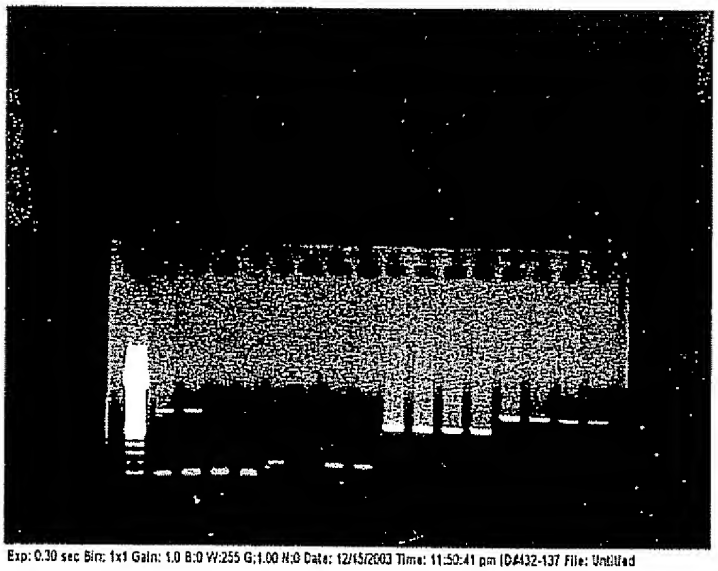
Extract genomic DNA from 200 µl blood sample
using Qiaamp DNA Blood Mini Kit
- follow the protocol.
rest of the blood: store at -80°C
→ elute w/ 200 µl buffer AE

Setup PCR reactions.

primers / template (genomic DNA 1 µL/rxn)	VKOR-G-353 / VKOR-G-1279-R	248 / 2626-R	4305 / 5051-R	VKOR-3 13124769-3'
001	(1)	(5)	(9)	(13)
002	(2)	(6)	(10)	(14)
003	(3)	(7)	(11)	(15)
patient DNA (10/24/03) 45ng/µl	(4)	(8)	(12)	(16)

			x 20
5 µL	10X buffer	(w/ Mg^{2+})	100
1 µL	dNTP (10mM)		20
1 µL	DNA		
0.5 µL	primer 1		
0.5 µL	primer 2		
41.5 µL	H_2O		930
0.5 µL	Taq		10

95°C 30" }
55°C 30" } 35 cycles.
72°C 2' }



Exp: 0.30 sec Bin: 1x1 Gain: 1.0 B:0 W:255 G:1.00 H:0 Date: 12/15/2003 Time: 11:50:41 pm ID:4432-137 File: Untitled

TAB 5

12/17/2003

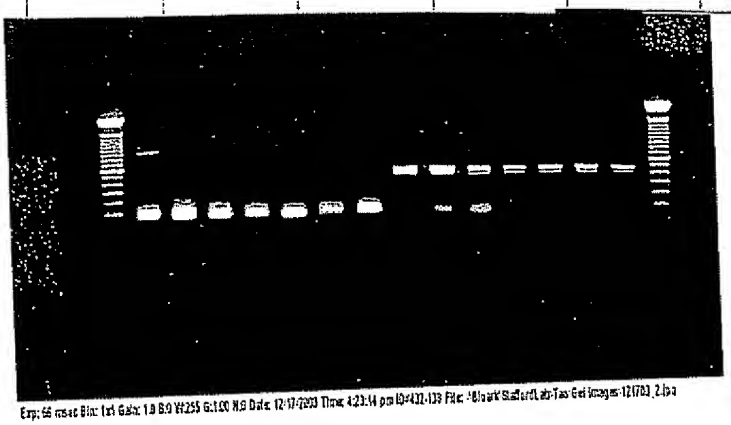
Do PCR:

Primers	Sample #001	002	003	004	005	006	007
Exon 1	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Exon 2	(8)	(9)	(10)	(11)	(12)	(13)	(14)
Exon 3	(15)	(16)	(17)	(18)	(19)	(20)	(21)

10x PCR buffer w/ Mg^{++}	5 µl
10 mM dNTP	1 µl
primer mix (50 µM)	1 µl
genomic DNA	4 µl
H_2O	38.5 µl
Taq	0.5 µl

95°C 30" }
60°C 30" } 40 cycles
72°C 1.5' }

1 ~ 14



15 ~ 21

good!

purify the PCR products using QiaKit

finally elute with 30 μl EB.

label as PCR#1 ~ PCR#21

Sequence samples#		template		primer (2 μM 5 μL)
blood DNA #001	①	PCR#1	8 μl	1279-R
	②	#8	8 μl	2148
	③	#8	8 μl	2626-R
	④	#15	2 μl	4305
	⑤	#15	2 μl	5051-R
#002	⑥	#2	8 μl	same order
	⑦	#9	8 μl	
	⑧	#9	8 μl	
	⑨	#16	2 μl	
	⑩	#16	2 μl	
#003	⑪	#3	8 μl	
	⑫	#10	8 μl	
	⑬	#10	8 μl	
	⑭	#17	2 μl	
	⑮	#17	2 μl	
#004	⑯	#4	8 μl	
	⑰	#11	8 μl	
	⑱	#11	8 μl	
	⑲	#18	2 μl	
	⑳	#18	2 μl	
#005	㉑	#5	8 μl	
	㉒	#12	8 μl	
	㉓	#12	8 μl	
	㉔	#19	2 μl	
	㉕	#19	2 μl	

#006	26	#6 8μl
	27	#13 8μl
	28	#13 8μl
	29	#20 2μl
	30	#20 2μl
#007	31	#7 8μl
	32	#14 8μl
	33	#14 8μl
	34	#21 2μl
	35	#21 2μl

TAB 6

Version 031003

Big Dye Termination Sequencing Required

UNC-CH Automated DNA Sequencing Facility
Rm. 126 Glaxo Bldg., CB# 7100
Tel. # 919-966-3763 FAX 966-6821
E-mail: dnaseq@med.unc.edu
WEB site: dnaseq.med.unc.edu

Cost per reaction (success or failure) including web distribution of data:

$10 UNC-CH: effective 8/12/03 submission
$28 other universities: effective 1/19/99 submission
$32 all others: effective 1/19/99 submission

Submission Date: 12/18/2003

First Name & Last Name of USER: Tao Li

First Name & Last Name of PI: Darrel Stafford

Dept. & CB# (UNC) or Inst.: Biology

Telephone #: 962-2267

The above information must be complete & legible for your order to be processed.

Check Blocks for Special Processing: ☐ Provide prints ($1.00/sample charge)

Off campus users: write address on top of page if you want prints sent by US mail.

- Samples will be tracked by submission date & sample ID number. (i.e. Dec 9 submission with sample ID's of 1-3 will be coded 1209001 through 1209003)
- Write your name (or an identifiable truncation), the date & the sample ID number on the top of each sample tube.

Make a copy of this submission sheet for your records BEFORE you turn it in. A copy will NOT be provided.

Complete the information below for each sample.

Please Do NOT Write In the Sections Below

Print on: _____ rxn on: _____ rx (in): _____

Work done: _____ Prt/Dsk: ___/___ Billed: _____

Run Date ___/___ uL/tot # Ln Rx# CycleSeq ID: _____

Sample ID Use numbers only!	DNA name (for your record)	PRIMER name (for your record)	Length of read needed		
0 1. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
0 2. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
0 3. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
0 4. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
0 5. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
0 6. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
0 7. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
0 8. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
0 9. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
10. (3 # max)	Name:	Name:	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt

Version 031003

Big Dye Terminator Sequence request

UNC-CH Automated DNA Sequencing Facility
Rm. 128 Glaxo Bldg., CB# 7100
Tel. # 919-966-3783 FAX 966-8821
E-mail: dnaseq@med.unc.edu
WEB site: dnaseq.med.unc.edu

Cost per reaction (success or failure) including web distribution of data:
$10 UNC-CH: effective 8/12/03 submission
$28 other universities: effective 1/19/99 submission
$32 all others: effective 1/19/99 submission

Submission Date: _____
First Name & Last Name of USER: Tao Li
First Name & Last Name of PI: _____
Dept. & CB# (UNC) or Inst.: _____
Telephone #: _____
The above information must be complete & legible for your order to be processed.

Check Blocks for Special Processing: ☐ Provide prints ($1.00/sample charge)
Off campus users: write address on top of page if you want prints sent by US mail.

- Samples will be tracked by submission date & sample ID number. (i.e. Dec 9 submission with sample ID's of 1-5 will be coded 1209001 through 1209005)
- Write your name (or an identifiable truncation), the date & the sample ID number on the top of each sample tube.

Make a copy of this submission sheet for your records BEFORE you turn it in. A copy will NOT be provided.

Complete the information below for each sample.

Please Do NOT Write in the Sections Below
Print ch: _____ rxn ch: _____ rx (in): _____
Work done: _____
Prt/Dak: _____/_____
Billed: _____
Run Date ___/___ uUtot # Ln RxBa CycleSeq ID: _____

Sample ID (Use numbers only!)	DNA name (for your record)	PRIMER name (for your record)	Length of read needed		
_1_ 1. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
_2_ 2. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
_3_ 3. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
_4_ 4. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
_5_ 5. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
_6_ 6. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
_7_ 7. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
_8_ 8. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
_9_ 9. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
20. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt

Version 031003

Dye Terminator Sequencing Reaction	UNC-CH Automated DNA Sequencing Facility Rm. 126 Glaxo Bldg., CB# 7100 Tel. # 919-966-3783 FAX 966-6821 E-mail: dnaseq@med.unc.edu WEB site: dnaseq.med.unc.edu

Cost per reaction (success or failure) including web distribution of data:

$10 UNC-CH: effective 6/12/03 submission
$28 other universities: effective 1/19/99 submission
$32 all others: effective 1/19/99 submission

Submission Date: _____

First Name & Last Name of USER: Tao Li

First Name & Last Name of PI: _____

Dept. & CB# (UNC) or Inst.: _____

Telephone #: _____

The above information must be complete & legible for your order to be processed.

Check Blocks for Special Processing: ☐ Provide prints ($1.00/sample charge)
Off campus users: write address on top of page if you want prints sent by US mail.

- Samples will be tracked by submission date & sample ID number. (i.e. Dec 9 submission with sample ID's of 1-5 will be coded 1209001 through 1209005)
- Write your name (or an identifiable truncation), the date & the sample ID number on the top of each sample tube.

Make a copy of this submission sheet for your records BEFORE you turn it in. A copy will NOT be provided.

Complete the information below for each sample.

Please Do NOT Write in the Sections Below

Print on: _____ rxn on: _____ rx (in): _____

Work done: _____
Prt/Dak: ___/___
Billed: _____

Run Date ___/___ uL/tot ___ # Ln ___ RxEx ___ CycleSeq ID: _____

Sample ID Use numbers only!	DNA name (for your record)	PRIMER name (for your record)	Length of read needed		
2 1. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
2 2. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
2 3. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
2 4. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
2 5. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
2 6. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
2 7. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
2 8. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
2 9. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
3 0. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases		( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt

Version 031008

Big Dye Terminator sequencing required

UNC-CH Automated DNA Sequencing Facility
Rm. 128 Glaxo Bldg., CB# 7100
Tel. # 919-966-3763 FAX 966-6821
E-mail: dnaseq@med.unc.edu
WEB site: dnaseq.med.unc.edu

Cost per reaction (success or failure) including web distribution of data:

$10 UNC-CH: effective 8/12/03 submission
$28 other universities: effective 1/19/99 submission
$32 all others: effective 1/19/99 submission

Submission Date: _____

First Name & Last Name of USER: Tao Li

First Name & Last Name of PI: _____

Dept. & CB# (UNC) or Inst.: _____

Telephone #: _____

The above information must be complete & legible for your order to be processed.

Check Blocks for Special Processing: ☐ Provide prints ($1.00/sample charge)
Off campus users: write address on top of page if you want prints sent by US mail.

- Samples will be tracked by submission date & sample ID number. (i.e. Dec 9 submission with sample ID's of 1-3 will be coded 1209001 through 1209003)
- Write your name (or an identifiable truncation), the date & the sample ID number on the top of each sample tube.

Make a copy of this submission sheet for your records BEFORE you turn it in. A copy will NOT be provided.

Complete the information below for each sample.

Please Do NOT Write in the Sections Below

Print on: _____ rxn on: _____ rx (in): _____

Work done: _____
Prt/Dsk: ___/___
Billed: _____

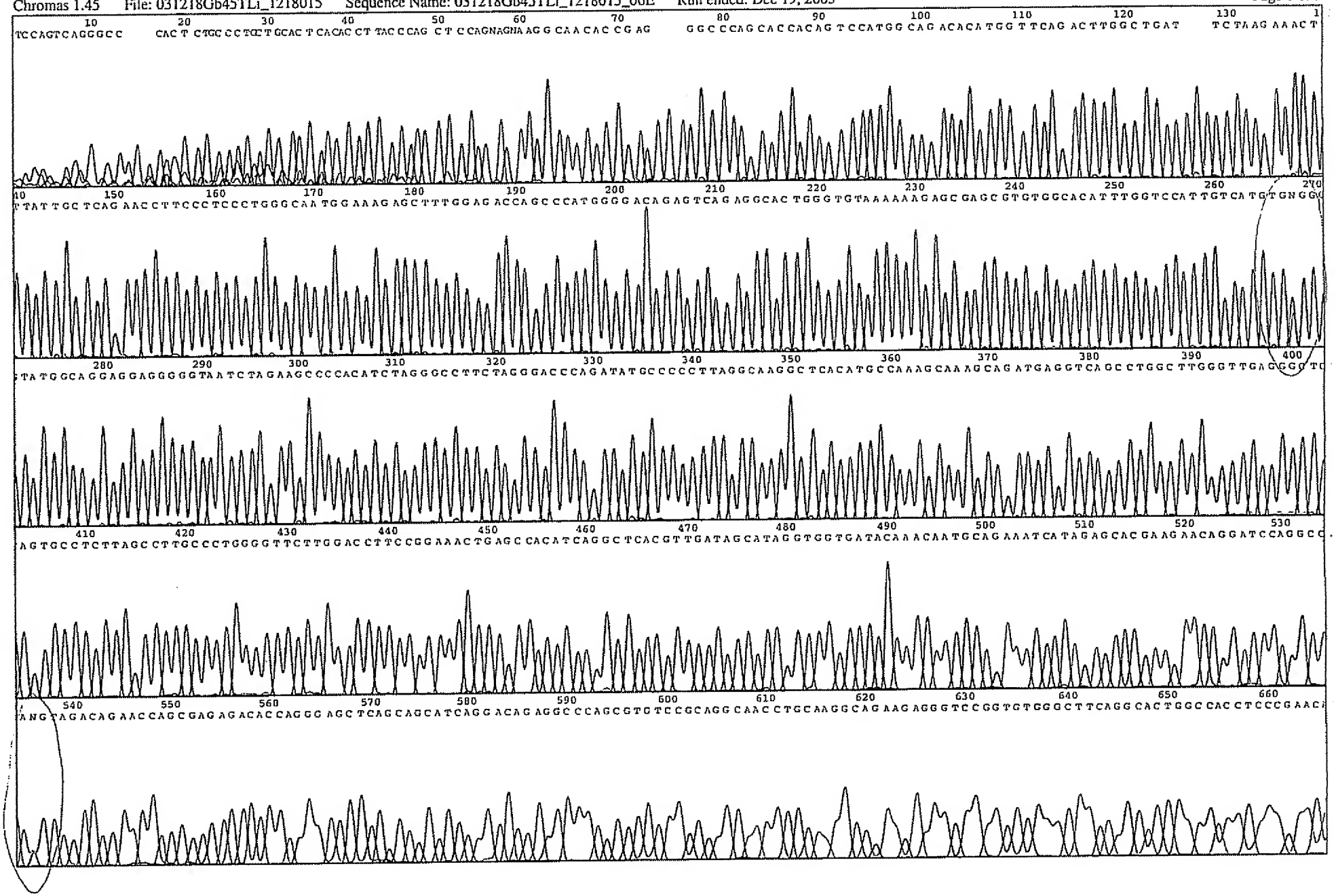
___/___ Run Date ___ uL/tot ___ # Ln ___ RxRx

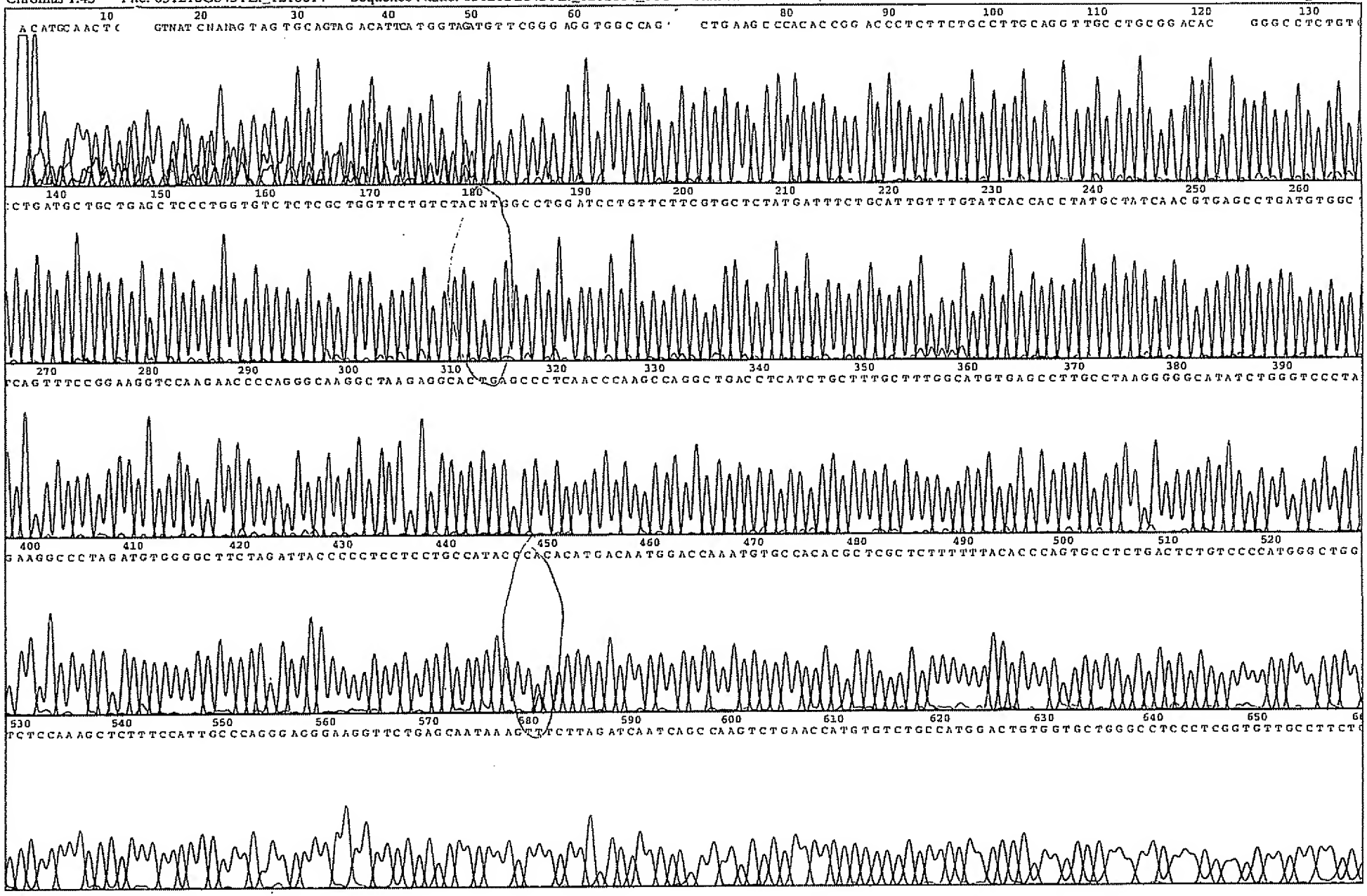
CycleSeq ID: _____

Sample ID Use numbers only	DNA name (for your record)	PRIMER name (for your record)	Length of read needed	
3_1. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
3_2. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
3_3. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
3_4. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
3_5. (3 # max)	Name: _____	Name: _____	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
__6. (3 # max)	Name: _____	Name: _____	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
__7. (3 # max)	Name: _____	Name: _____	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
__8. (3 # max)	Name: _____	Name: _____	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
__9. (3 # max)	Name: _____	Name: _____	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
__0. (3 # max)	Name: _____	Name: _____	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt

Submission ID:

TAB 7





003 E3: V Y L A

GTCTACCTGGCC

↓

T

L

hetero-

007 E3 ATACCCGCACA

003 ↓

004 A

hetero-

003 E2 ...CCGCCCCCCTCCCT

G

TAB 8

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Fri, 19 Dec 2003 22:26:51 -0500 (Eastern Standard Time)
Subject: Re: samples
To: Lisa_Gatti@med.unc.edu

Hi! Lisa,

The sequencing facility is pretty slow in holiday season, besides, they will close from next Monday to January 2. So let's try to collect the next batch of samples after Jan 2. The sequence results of the first 3 samples should come out by the end of today. If I find interesting results, I'll tell you.

Have a nice holiday!

Tao

-- Begin original message --

> From: "Lisa R. Gatti" <lgatti@med.unc.edu>
> Date: Fri, 19 Dec 2003 12:19:50 -0500
> Subject: Re: samples
> CC: taoli@email.unc.edu
> Reply-To: Lisa_Gatti@med.unc.edu
>
> Tao,
>
> As it turns out, there will not be any samples today. I will not be
> recruiting again until Tuesday 12/30. Do you want any samples on 12/31,
> or will you be closed?
>
> Have a great holiday!
> Lisa G.
>

-- End original message --

TAB 9

Printed by: Tao Li

Jan 06, 2004

From: taoli@email.unc.edu (Tao Li)
Date: Tue, 6 Jan 2004 15:28:22 -0500 (Eastern Standard Time)
Subject: Re: amount of blood
To: Lisa_Susswein@med.unc.edu ("Susswein, Lisa")
Cc: jpevans@med.unc.edu, dws@email.unc.edu

Hi! Lisa,

2x 3 mL tubes are quite enough for me. Thanks! Actually I only use 200 uL blood to extract genomic DNAs and store the rest in -80 degree.

I chatted with Lisa Gatti for a while yesterday when I picked up the blood. So far I have sequenced 7 samples. One of them has a "C" to "T" mutation in the coding region, however, it's Leu to Leu. Three out of seven have "G" to "A" mutations in the 3'-UTR, which may or may not mean anything. Both of the 2 kinds of mutations are heterozygous. Because I have not detected any mutations that cause the amino acid changing as we expected , I am not very excited at this time. :-) But at least we have seen something. I believe after we sequence more samples, we can see more polymorphisms.

Have a nice day!

Tao

-- Begin original message --

> From: "Susswein, Lisa" <Lisa_Susswein@med.unc.edu>
> Date: Tue, 6 Jan 2004 13:31:49 -0500
> Subject: amount of blood
> To: "'Tao Li'" <taoli@email.unc.edu>
>
>
> ------_=_NextPart_001_01C3D482.EF2522F2
> Content-Type: text/plain
>
> Hey Tao,
>
>
>
> I hear from Lisa that you have some exciting news! Some polymorphisms? I
> wanted to ask about the amount of blood you need. I've discovered that the
> 3ml tubes are much cheaper than 6ml. Would 2x 3 ml tubes (6 ml total) be
> enough? Right now we are getting 2x 6ml tubes (12 total).
>
>
>
> Thanks,
>
> Lisa
>

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Tue, 6 Jan 2004 15:53:35 -0500 (Eastern Standard Time)
Subject: Re: RE: amount of blood
To: jpevans@med.unc.edu ("Evans, Jim")

Hi! Jim,

You are absolutely right. I've read that paper before. It's not so straightforward to understand 3'-UTR mutations. We have to do more experiments to study it, for example, transfect a construct containing the mutation and test the mRNA stability. I'll keep it in mind that UTR mutations may also be important. Thanks!

Have a nice day!

Tao

-- Begin original message --

> From: "Evans, Jim" <jpevans@med.unc.edu>
> Date: Tue, 6 Jan 2004 15:27:50 -0500
> Subject: RE: amount of blood
> To: "'taoli@email.unc.edu'" <taoli@email.unc.edu>
>
> Hey Tao,
>
> Don't discount the 3' (or 5') UT region changes. A very important
> polymorphism in coagulation, the prothrombin 20210 change, I believe, is in
> the 3'UT region and is very important clinically!
>
> Jim
>

TAB 10

01/08/2004

purify the PCR products using Qiakit
elute w/ 30μl EB each

Label as:

#008 ~ #018
PCR
Exon 3
01/08

② Prepare sequencing samples.

2μl PCR product / sample
primer: VKOR-G-4305 & VKOR-G-5051-R
5μl 2μM
13μl ddH2O

(1)	#008	primer: VKOR-G-4305
(2)	009	
(3)	010	
(4)	011	
(5)	012	
(6)	013	
(7)	014	
(8)	015	
(9)	016	
(10)	017	
(11)	018	
(12)	#008	primer: VKOR-G-5051-1
(13)	009	
(14)	010	
(15)	011	
(16)	012	
(17)	013	
(18)	014	
(19)	015	
(20)	016	
(21)	017	
(22)	018	

Version 031008

Big Dye Terminator cycle sequencing required

UNC-CH Automated DNA Sequencing Facility
Rm. 128 Glaxo Bldg., CB# 7100
Tel. # 919-966-3783 FAX 966-6821
E-mail: dnaseq@med.unc.edu
WEB site: dnaseq.med.unc.edu

Cost per reaction (success or failure) including web distribution of data:
$10 UNC-CH: effective 8/12/03 submission
$28 other universities: effective 1/19/99 submission
$32 all others: effective 1/19/99 submission

Submission Date: 01/08/2004
First Name & Last Name of USER: Tao Li
First Name & Last Name of PI: Darrel Stafford
Dept. & CB# (UNC) or Inst.: Biology
Telephone #: 962-2267
The above information must be complete & legible for your order to be processed.

Check Blocks for Special Processing: ☐ Provide prints ($1.00/sample charge)
Off campus users: write address on top of page if you want prints sent by US mail.

- Samples will be tracked by submission date & sample ID number. (I.e, Dec 9 submission with sample ID's of 1-5 will be coded 1209001 through 1209005)
- Write your name (or an identifiable truncation), the date & the sample ID number on the top of each sample tube.

Make a copy of this submission sheet for your records BEFORE you turn it in. A copy will NOT be provided.

Complete the information below for each sample.

Please Do NOT Write in the Sections Below
Print ch: _____ rxn ch: _____ rx (in): _____
Work done: _____
Prt/Dak: ____/____
Billed: _____
Run Date ____/____ uL/tot ____ # Ln ____ RxBs ____
CycleSeq ID: _____

Sample ID (Use numbers only)	DNA name (for your record)	PRIMER name (for your record)	Length of read needed	
01. (3 # max)	Name: 008	Name: G4305	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
02. (3 # max)	Name: 009	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
03. (3 # max)	Name: 010	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
04. (3 # max)	Name: 011	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
05. (3 # max)	Name: 012	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
06. (3 # max)	Name: 013	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
07. (3 # max)	Name: 014	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
08. (3 # max)	Name: 015	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
09. (3 # max)	Name: 016	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
10. (3 # max)	Name: 017	Name: ↓	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt

Version 031003

Big Dye Terminator sequencing reagent

UNC-CH Automated DNA Sequencing Facility
Rm. 128 Glaxo Bldg., CB# 7100
Tel. # 919-966-3783 FAX 966-6821
E-mail: dnaseq@med.unc.edu
WEB site: dnaseq.med.unc.edu

Cost per reaction (success or failure) including web distribution of data:

$10 UNC-CH: effective 8/12/03 submission
$28 other universities: effective 1/19/99 submission
$32 all others: effective 1/19/99 submission

Submission Date: _____

First Name & Last Name of USER: Tao Li

First Name & Last Name of PI: _____

Dept. & CB# (UNC) or Inst.: _____

Telephone #: _____

The above information must be complete & legible for your order to be processed.

Check Blocks for Special Processing: ☐ Provide prints ($1.00/sample charge)

Off campus users: write address on top of page if you want prints sent by US mail.

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Complete the information below for each sample.

Please Do NOT Write in the Sections Below

Print ch: _____ rxn ch: _____ Work done: _____

rx (in): _____ Prt/Dak: ____/____

Billed: _____

Run Date ____/____ uL/rxt ____ # Ln ____ RxSz ____ CycleSeq ID: _____

Sample ID (Use numbers only)	DNA name (for your record)	PRIMER name (for your record)	Length of read needed	
_1_ 1. (3 # max)	Name: 018	Name: G-4305	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
_2_ 2. (3 # max)	Name: 008	Name: G-5051-R	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
_3_ 3. (3 # max)	Name: 009	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
_4_ 4. (3 # max)	Name: 010	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
_5_ 5. (3 # max)	Name: 011	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
_6_ 6. (3 # max)	Name: 012	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
_7_ 7. (3 # max)	Name: 013	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
_8_ 8. (3 # max)	Name: 014	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
_9_ 9. (3 # max)	Name: 015	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt
20. (3 # max)	Name: 016	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt

 Submission ID:

Version 031008

Big Dye Terminator Sequencing required	UNC-CH Automated DNA Sequencing Facility Rm. 128 Glaxo Bldg., CB# 7100 Tel. # 919-966-3783 FAX 966-6821 E-mail: dnaseq@med.unc.edu WEB site: dnaseq.med.unc.edu

Cost per reaction (success or failure) including web distribution of data:
- $10 UNC-CH: effective 6/12/03 submission
- $28 other universities: effective 1/19/99 submission
- $32 all others: effective 1/19/99 submission

Submission Date: _____

First Name & Last Name of USER: Tao Li

First Name & Last Name of PI: _____

Dept. & CB# (UNC) or Inst.: _____

Telephone #: _____

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Complete the information below for each sample.

Please Do NOT Write in the Sections Below

Print ch: _____ rxn ch: _____ rx (ln): _____

Work done: _____

Prt/Dsk: ____/____

Billed: _____

Run Date ___/___ uL/tot ___ # Ln ___ Rxns ___

CycleSeq ID: _____

Sample ID Use numbers only!	DNA name (for your record)	PRIMER name (for your record)	Length of read needed	
1. (3 # max)	Name: 018	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
2. (3 # max)	Name: 018	Name:	☒ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
3. (3 # max)	Name:	Name:	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
4. (3 # max)	Name:	Name:	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
5. (3 # max)	Name:	Name:	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
6. (3 # max)	Name:	Name:	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
7. (3 # max)	Name:	Name:	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
8. (3 # max)	Name:	Name:	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
9. (3 # max)	Name:	Name:	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt
0. (3 # max)	Name:	Name:	☐ or ☐ ____ Max # bases	( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt ( ) Done ( ) LnRpt ( ) RxnRpt

Submission ID:

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014-E3                    GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
008-E3                    GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
015-E3                    GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
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017-E3                    GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
009-E3                    GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
010-E3                    GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
011-E3                    GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
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013-E3-R                  GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
008-E3-R                  GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
009-E3-R                  GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
012-E3-R                  GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
010-E3-R                  GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
015-E3-R                  GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
016-E3-R                  GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
017-E3-R                  GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
VKOR_genomic_DNA          GCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACCTCATCTGCTTTGCTT
                          ************************************************************

018-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
014-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
008-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
015-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
013-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
016-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
017-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
009-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
010-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
011-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
012-E3                    TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
014-E3-R                  TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
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013-E3-R                  TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
008-E3-R                  TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
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012-E3-R                  TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
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016-E3-R                  TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
017-E3-R                  TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
VKOR_genomic_DNA          TGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGATGTGGG
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                                                                    4789
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009-E3                    GCTTCTAGATTACCCCCTCCTCCTGCCATACCCACACATGACAATGGACCAAATGTGCCA
010-E3                    GCTTCTAGATTACCCCCTCCTCCTGCCATACCCGCACATGACAATGGACCAAATGTGCCA
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012-E3                    GCTTCTAGATTACCCCCTCCTCCTGCCATACCCACACATGACAATGGACCAAATGTGCCA
014-E3-R                  GCTTCTAGATTACCCCCTCCTCCTGCCATACCCGCACATGACAATGGACCAAATGTGCCA
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013-E3-R                  GCTTCTAGATTACCCCCTCCTCCTGCCATACCCACACATGACAATGGACCAAATGTGCCA
008-E3-R                  GCTTCTAGATTACCCCCTCCTCCTGCCATACCCGCACATGACAATGGACCAAATGTGCCA
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012-E3-R                  GCTTCTAGATTACCCCCTCCTCCTGCCATACCCACACATGACAATGGACCAAATGTGCCA
010-E3-R                  GCTTCTAGATTACCCCCTCCTCCTGCCATACCCGCACATGACAATGGACCAAATGTGCCA
015-E3-R                  GCTTCTAGATTACCCCCTCCTCCTGCCATACCCNCACATGACAATGGACCAAATGTGCCA
016-E3-R                  GCTTCTAGATTACCCCCTCCTCCTGCCATACCCGCACATGACAATGGACCAAATGTGCCA
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017-E3-R            GCTTCTAGATTACCCCCTCCTCCTGCCATACCCNCACATGACAATGGACCAAATGTGCCA
VKOR_genomic_DNA    GCTTCTAGATTACCCCCTCCTCCTGCCATACCCGCACATGACAATGGACCAAATGTGCCA
                    ********************************* ************************

`18-E3              CACGCTCGCTCTTTTTTACACCCAGTGCCTCTGACTCTGTCCCCATGGGCTGGTCTCCAA
 14-E3              CACGCTCGCTCTTTTTTACACCCAGTGCCTCTGACTCTGTCCCCATGGGCTGGTCTCCAA
008-E3              CACGCTCGCTCTTTTTTACACCCAGTGCCTCTGACTCTGTCCCCATGGGCTGGTCTCCAA
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017-E3-R            CACGCTCGCTCTTTTTTACACCCAGTGCCTCTGACTCTGTCCCCATGGGCTGGTCTCCAA
VKOR_genomic_DNA    CACGCTCGCTCTTTTTTACACCCAGTGCCTCTGACTCTGTCCCCATGGGCTGGTCTCCAA
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018-E3              AGCTCTTTCCATTGCCCAGGGAGGGAAGGTTCTGAGCAATAAAGTTTCTTAGATCAATCA
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016-E3              AGCTCTTTCCATTGCCCAGGGAGGGAAGGTTCTGAGCAATAAAGTTTCTTAGATCAATCA
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016-E3-R            AGCTCTTTCCATTGCCCAGGGAGGGAAGGTTCTGAGCAATAAAGTTTCTTAGATCAATCA
017-E3-R            AGCTCTTTCCATTGCCCAGGGAGGGAAGGTTCTGAGCAATAAAGTTTCTTAGATCAATCA
VKOR_genomic_DNA    AGCTCTTTCCATTGCCCAGGGAGGGAAGGTTCTGAGCAATAAAGTTTCTTAGATCAATCA
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018-E3              GCCAAGTCTGAACCATGTGTCTGCCATGGACTGTGGTGCTGGGCCTCCCTCGGTGTTGCC
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009-E3              GCCAAGTCTGAACCATGTGTCTGCCATGGACTGTGGTGCTGGGCCTCCCTCGGTGTTGCC
010-E3              GCCAAGTCTGAACCATGTGTCTGCCATGGACTGTGGTGCTGGGCCTCCCTCGGTGTTGCC
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014-E3-R            GCCAAGTCTGAACCATGTGTCTGCCATGGACTGTGGTGCTGGGCCTCCCTCGGTGTTGCC
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008-E3-R            GCCAAGTCTGAACCATGTGTCTGCCATGGACTGTGGTGCTGGGCCTCCCTCGGTGTTGCC
009-E3-R            GCCAAGTCTGAACCATGTGTCTGCCATGGACTGTGGTGCTGGGCCTCCCTCGGTGTTGCC
012-E3-R            GCCAAGTCTGAACCATGTGTCTGCCATGGACTGTGGTGCTGGGCCTCCCTCGGTGTTGCC
010-E3-R            GCCAAGTCTGAACCATGTGTCTGCCATGGACTGTGGTGCTGGGCCTCCCTCGGTGTTGCC
```

TAB 11

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Thu, 8 Jan 2004 10:52:22 -0500 (Eastern Standard Time)
Subject: Re: friday
To: Lisa_Gatti@med.unc.edu

OK, thanks, Lisa.

Tao

-- Begin original message --

> From: "Lisa R. Gatti" <lgatti@med.unc.edu>
> Date: Thu, 08 Jan 2004 09:23:40 -0500
> Subject: friday
> To: Tao Li <taoli@email.unc.edu>
> Reply-To: Lisa_Gatti@med.unc.edu
>
> Tao,
>
> I just wanted to warn you that tomorrow (Friday) might not be a very
> productive day in the clinic. It is supposed to snow tomorrow morning.
> The last time there was snow/ice in the forecast, over 1/2 of the
> scheduled patients did not attend their appointments. I will be very
> surprised if I am able to obtain enough samples to reach our goal of
> 24. However, there are always more Coumadin patients scheduled for
> Mondays, so hopefully next Monday will be as successful as this last one
> was.
>
> I will email/call you at some point during the day to let you know when
> I will bring the samples that I do get to you.
>
> Thanks!
> Lisa G.
>

-- End original message --

TAB 12

ATACCCGCACAT

↓

A

#009 Homozygous

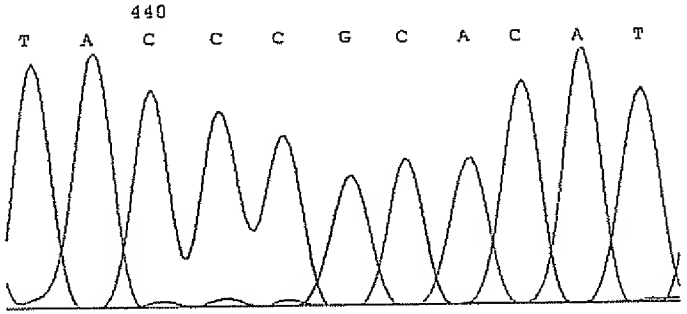
#013 Homozygous

#015 heterozygous

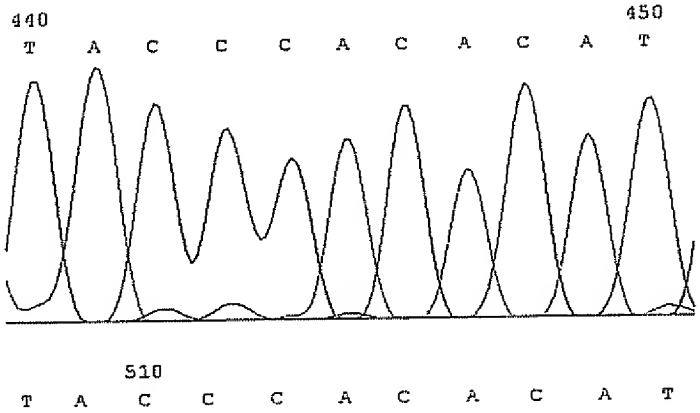
#017 heterozygous

VKOR 3'-UTR G>A plolymorphism: #009 & #013, homozygous
Sample date: 01/08/04 Analyze date: 01/12/04
Samples: #008~#018

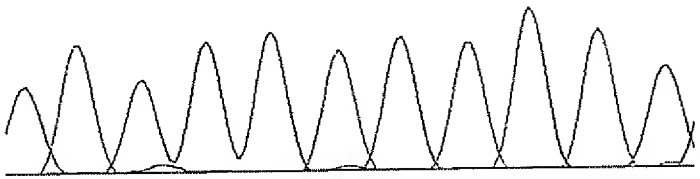
Wildtype



#009

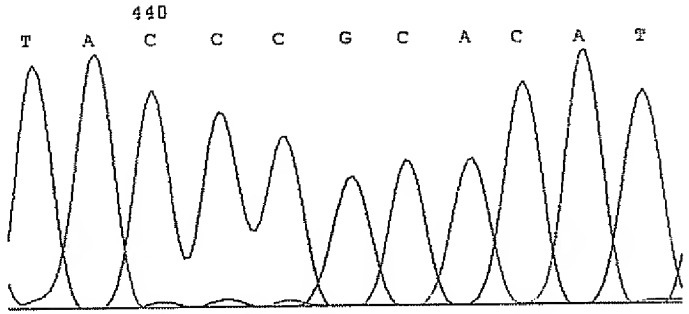


#009 reverse seq

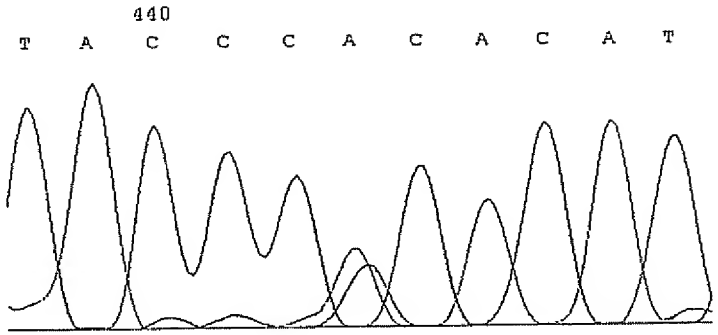


VKOR 3'-UTR G>A plolymorphism: #015 & #017, heterozygous
Sample date: 01/08/04 Analyze date: 01/12/04
Samples: #008~#018

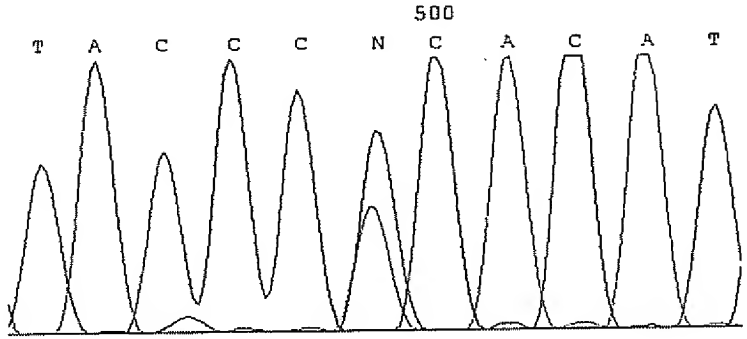
Wildtype



#015



#015 reverse seq



TAB 13

1/12/2004

pick up blood samples from Lisa Gatti
#19 . #20
extract genomic DNAs using QiaKit
finally dissolve in 200 µL AE.

1/13/2004

pick up blood samples from Lisa Gatti
#21 #22 #23
extract genomic DNAs using QiaKit
200 µL AE

Lisa Gatti, RN
Study Coordinator, Breast Cancer Research

UNC LINEBERGER COMPREHENSIVE CANCER CENTER

The University of North Carolina at Chapel Hill
Campus Box # 7295, MDC
Chapel Hill, NC 27599-7295

Phone (919) 843-5942
Fax (919) 966-0393
Pager (919) 216-2050

1/14/2004

blood sample #24 → extract genomic DNA

Send all 24 genomic DNA samples to Polymorpicdna.com

30 ng/µL x 30 µL / sample

POLYMORPHIC
Polymorphic DNA Technologies, Inc.

SNP Discovery Genomic Sequence Information Sheet

Institution name: University of North Carolina at Chapel Hill, Biology Dept., CB#3280, Chapel Hill, NC 27599
Contact name: Tao Li
Phone: 919-962-2267
Email: taoli@email.unc.edu

Purchase Order Number: K-284595

Region ID	Genomic Sequence	Chromosome number
13124769 Exon1	See attachment file "exon1.txt"	16
13124769 Exon2	See attachment file "exon2.txt"	16
13124769 Exon3	See attachment file "exon3.txt"	

POLYMORPHIC
Polymorphic DNA Technologies, Inc.

Institution name: University of North Carolina at Chapel Hill, Biology Dept., CB#3280, Chapel Hill, NC 27599
Contact name: Tao Li
Phone: 919-962-2267
Email: taoli@email.unc.edu

Plate Name: Tao #1~#24
Purification Method: QiaGen QIAamp Blood Mini Kit

Well Position	Sample ID	Volume (µL)	Concentration	Buffer Type
A01	1	30	30 ng/uL	AE
B01	2	30	30 ng/uL	AE
C01	3	30	30 ng/uL	AE
D01	4	30	30 ng/uL	AE
E01	5	30	30 ng/uL	AE
F01	6	30	30 ng/uL	AE
G01	7	30	30 ng/uL	AE
H01	8	30	30 ng/uL	AE
A02	9	30	30 ng/uL	AE
B02	10	30	30 ng/uL	AE
C02	11	30	30 ng/uL	AE
D02	12	30	30 ng/uL	AE
E02	13	30	30 ng/uL	AE
F02	14	30	30 ng/uL	AE
G02	15	30	30 ng/uL	AE
H02	16	30	30 ng/uL	AE
A03	17	30	30 ng/uL	AE
B03	18	30	30 ng/uL	AE
C03	19	30	30 ng/uL	AE
D03	20	30	30 ng/uL	AE

E03	21	30	30 ng/uL	AE
F03	22	30	30 ng/uL	AE
G03	23	30	30 ng/uL	AE
H03	24	30	30 ng/uL	AE
A04				
B04				
C04				
D04				
E04				
F04				
G04				
H04				
A05				
B05				
C05				
D05				
E05				
F05				
G05				
H05				
A06				
B06				
C06				
D06				
E06				
F06				
G06				
H06				
A07				
B07				
C07				
D07				
E07				
F07				
G07				
H07				

>13124769 Exon1

CGCCGTATTCGCTGGATCCCCTGATCCGCTGGTCTCTAGGTCCCGGATGCTGCAATTCTTACAACAGGAC
TTGGCATAGGGTAAGCGCAAATGCTGTTAACCACACTAACACACTTTTTTTTTTCTTTTTTTTTTTTTGAG
ACAGAGTCTCACTCTGTCGGCCTGGCTGGAGTGCAGTGGCACGATCTCGGCTCACTGCAACCTCCGGCTC
CCCGGCTCAAGCAATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGATTACAGGCATGTGCCACCACGCCC
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ACAGTTTTTAAATCTGTGGAGACTTCATTTCCCTTGATGCCTTGCAGCCGCGCCGACTACAACTCCCATC
ATGCCTGGCAGCCGCTGGGGCCGCGATTCCGCACGTCCCTTACCCGCTTCACTAGTCCCGGCATTCTTCG
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GGTTTTCTCCGCGGGCGCCTCGGGCGGAACCTGGAGATAATGGGCAGCACCTGGGGGAGCCCTGGCTGGG
TGCGGCTCGCTCTTTGCCTGACGGGCTTAGTGCTCTCGCTCTACGCGCTGCACGTGAAGGCGGCGCGCGC
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AGGTGTGCACGGGAGTGGGAGGCGTGGGGCCTCGGAGCAGGGCGGCCAGG

>13124769 Exon2

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Exon3

>13124769 Exon3

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CTGTCCTGATGCTGCTGAGCTCCCTGGTGTCTCTCGCTGGTTCTGTCTACCTGGCCTGGATCCTGTTCTT
CGTGCTCTATGATTTCTGCATTGTTTGTATCACCACCTATGCTATCAACGTGAGCCTGATGTGGCTCAGT
TTCCGGAAGGTCCAAGAACCCCAGGGCAAGGCTAAGAGGCACTGAGCCCTCAACCCAAGCCAGGCTGACC
TCATCTGCTTTGCTTTGGCATGTGAGCCTTGCCTAAGGGGGCATATCTGGGTCCCTAGAAGGCCCTAGAT
GTGGGGCTTCTAGATTACCCCCTCCTCCTGCCATACCCGCACATGACAATGGACCAAATGTGCCACACGC
TCGCTCTTTTTTACACCCAGTGCCTCTGACTCTGTCCCCATGGGCTGGTCTCCAAAGCTCTTTCCATTGC
CCAGGGAGGGAAGGTTCTGAGCAATAAAGTTTCTTAGATCAATCAGCCAAGTCTGAACCATGTGTCTGCC
ATGGACTGTGGTGCTGGGCCTCCCTCGGTGTTGCCTTCTCTGGAGCTGGGAAGGGTGAGTCAGAGGGAGA
GTGGAGGGCCTGCTGGGAAGGGTGGTTATG

TAB 14

rinted by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Wed, 14 Jan 2004 15:47:44 -0500 (Eastern Standard Time)
Subject: SNP samples: Tao Li (UNC)
To: info@polymorphicdna.com

Jan 14, 2004

Hi! Dear Mr. Keith,

It was nice to talk with you by phone last week. Here I am sending the gene information for SNP discovery. I have also sent the 24 genomic DNA samples to your company by FedEX overnight. If the first 24 samples works well, we'll do the rest of hundreds of samples in your company. Thank you very much! Look forward to hearing from you soon.

Have a nice day!

Tao

Tao Li, PhD
438 Wilson Hall, CB#3280
Biology Dept.
University of North Carolina at Chapel Hill
Chapel Hill, NC 27514
Tel: 919-962-2267
Fax: 919-962-9266

There are 5 attachments in this message:

Attachment 1 - 280654 bytes
    Content Type : APPLICATION/MSEXCEL

Attachment 2 - 49820 bytes
    Content Type : APPLICATION/MSEXCEL

Attachment 3 - 1007 bytes
    Content Type : TEXT/PLAIN

Attachment 4 - 268 bytes
    Content Type : TEXT/PLAIN

Attachment 5 - 699 bytes
    Content Type : TEXT/PLAIN

POLYMORPHIC
Polymorphic DNA Technologies, Inc.

Institution name: University of North Carolina at Chapel Hill, Biology Dept., CB#3280, Chapel Hill, NC 27599
Contact name: Tao Li
Phone: 919-962-2267
Email: taoli@email.unc.edu

Plate Name: Tao #1~#24
Purification Method: QiaGen QIAamp Blood Mini Kit

Well Position	Sample ID	Volume (µL)	Concentration	Buffer Type
A01	1	30	30 ng/uL	AE
B01	2	30	30 ng/uL	AE
C01	3	30	30 ng/uL	AE
D01	4	30	30 ng/uL	AE
E01	5	30	30 ng/uL	AE
F01	6	30	30 ng/uL	AE
G01	7	30	30 ng/uL	AE
H01	8	30	30 ng/uL	AE
A02	9	30	30 ng/uL	AE
B02	10	30	30 ng/uL	AE
C02	11	30	30 ng/uL	AE
D02	12	30	30 ng/uL	AE
E02	13	30	30 ng/uL	AE
F02	14	30	30 ng/uL	AE
G02	15	30	30 ng/uL	AE
H02	16	30	30 ng/uL	AE
A03	17	30	30 ng/uL	AE
B03	18	30	30 ng/uL	AE
C03	19	30	30 ng/uL	AE
D03	20	30	30 ng/uL	AE

E03	21	30	30 ng/uL	AE
F03	22	30	30 ng/uL	AE
G03	23	30	30 ng/uL	AE
H03	24	30	30 ng/uL	AE
A04				
B04				
C04				
D04				
E04				
F04				
G04				
H04				
A05				
B05				
C05				
D05				
E05				
F05				
G05				
H05				
A06				
B06				
C06				
D06				
E06				
F06				
G06				
H06				
A07				
B07				
C07				
D07				
E07				
F07				
G07				
H07				
A08				

POLYMORPHIC
Polymorphic DNA Technologies, Inc.

SNP Discovery Genomic Sequence Information Sheet

Institution name: University of North Carolina at Chapel Hill, Biology Dept., CB#3280, Chapel Hill, NC 27599
Contact name: Tao Li
Phone: 919-962-2267
Email: taoli@email.unc.edu

Purchase Order Number: K-284595

Region ID	Genomic Sequence	Chromosome number
13124769 Exon1	See attachment file "exon1.txt"	16
13124769 Exon2	See attachment file "exon2.txt"	16
13124769 Exon3	See attachment file "exon3.txt"	

TAB 15

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Fri, 16 Jan 2004 15:56:22 -0500 (Eastern Standard Time)
Subject: Re: Any samples today?
To: Lisa_Gatti@med.unc.edu
Cc: Lisa_Susswein@med.unc.edu, jpevans@med.unc.edu, dws@email.unc.edu

Hi! Lisa,

The sequence results for the first 24 samples will come out in 7-10 business days. The reason that I sent the samples to the company instead of UNC sequence facility is that the company charge 70% less for batch sequencing, besides, they can design more effective primers for genomic sequencing. I ordered 3 pairs of primers for the 3 exons, and all of them can work, however, 2 pair of primers cause high sequencing background which affects the reading of heterozygous polymorphisms. So I only did partial sequencing for the first 18 samples and let the company do the rest for me.

Here is the summary of the preliminary data from partial sequencing out of 18 patients:
1. C>T mutation in exon 3 (Leu>Leu): 1 patient, heterozygous
2. G>A mutation in 3'-UTR: 5 patients, heterozygous; 2 patients, homozygous

It's possible that these mutations are related to the phenotype and it's also possible that we can detect some other polymorphisms in exon1, exon2 and 5'-UTR.

Because collecting blood samples is a long process (ave. 2-4 patients/day, 10 patients/week), I suggest we keep on blood drawing while we are waiting for the results. In that case, if we can have positive results for the first 24, we can immediately send out the next 24 samples and save time.

Jim and Lisa Susswein, how do you think?

Have a nice weekend!

Tao

-- Begin original message --

> From: "Lisa R. Gatti" <lgatti@med.unc.edu>
> Date: Fri, 16 Jan 2004 15:24:32 -0500
> Subject: Re: Any samples today?
> To: Tao Li <taoli@email.unc.edu>
> Reply-To: Lisa_Gatti@med.unc.edu
>
> Tao,
>
> I will not be collecting any samples today.... we are going to wait
> for the results from the first 24 to come in. Then I think we will
> regroup and then continue. Unless I hear differently from Lisa
> Susswein, I will not be recruiting. Please let me know when the results
> have come in, and I'll definitely let you know if Lisa/Jim want me to
> start recruiting again in the meantime.
> Thanks!- Have a nice weekend!
> Lisa g.
>
>
>
> Tao Li wrote:
> >
> > Hi! Lisa,
> >
> > Are you going to collect any samples today?
> >
> > Tao
>

-- End original message --

TAB 16

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Tue, 24 Feb 2004 14:12:36 -0500 (Eastern Standard Time)
Subject: Re: this week
To: Lisa_Gatti@med.unc.edu

Hi! Lisa,

I'll go pick up the samples around 4pm. Please give me a call when you are ready. Thanks!

Tao

-- Begin original message --

> From: "Lisa R. Gatti" <lgatti@med.unc.edu>
> Date: Tue, 24 Feb 2004 13:19:22 -0500
> Subject: Re: this week
> To: Tao Li <taoli@email.unc.edu>
> Reply-To: Lisa_Gatti@med.unc.edu
>
> tao,
> i will be finished obtaining samples by 4:00 today- but won't be
> leaving until much later (6:00ish). So, if you want the samples
> earlier- I can meet you at the Cancer Center anytime after 4:00 -
> otherwise, i can meet you at the bus-stop on my way out.
> let me know what works best for you.
> thanks,
> lisa g
>
>

TAB 17

Printed by: Tao Li Feb 26, 2004

From: taoli@email.unc.edu (Tao Li)
Date: Thu, 26 Feb 2004 15:55:18 -0500 (Eastern Standard Time)
Subject: VKOR SNP result of the 24 patients
To: jpevans@med.unc.edu ("Evans, Jim")
Cc: dws@email.unc.edu ("'dws@email.unc.edu '"),
Lisa_Susswein@med.unc.edu ("Susswein, Lisa"),
Lisa_Gatti@med.unc.edu ("'Lisa_Gatti@med.unc.edu '")

Hi! Jim,

The VKOR SNP result has come out. Sorry about the delay. The company got a bad batch of primers and they spent a period time to figure it out, so the result was delayed for about 3 weeks.

Here is the summary of VKOR SNPs for the first 24 patients:

1 out of 24 has 563 G>A (5'-UTR), heterozygous.
1 out of 24 has 4501 C>T (exon3, Leu120Leu), heterozygous.
11 out of 24 have 4769 G>A (3'-UTR). Among them, 8 patients are heterozygous and 3 are homozygous.

Maybe we can pick a time to have a group meeting recently. Lisa Susswein, could you please help to set up the meeting? Let's see if there is any correlation between SNP results and patient data.

So far we have 43 patient blood samples. Lisa Gatti, thank you for your efforts to collect them. If we send another 24 samples to the company, they can do the SNP test much faster than the first time.

Have a nice day!

Tao

TAB 18

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Tue, 2 Mar 2004 18:14:27 -0500 (Eastern Standard Time)
Subject: Tao Li's samples #25-#48
To: keith@polymorphicdna.com (Keith Williamson)

Hi! Keith,

Here is the list of my samples #25-#48. I will ship the samples to you using FedEx tomorrow. Please use purchase order# K-307831 for this batch. Thanks!

Regards,

Tao

Biology Dept.
University of North Carolina at Chapel Hill
919-360-8663 (cell)

There are 5 attachments in this message:

Attachment 1 - 49820 bytes
Content Type : APPLICATION/MSEXCEL

Attachment 2 - 280654 bytes
Content Type : APPLICATION/MSEXCEL

Attachment 3 - 1007 bytes
Content Type : TEXT/PLAIN

Attachment 4 - 268 bytes
Content Type : TEXT/PLAIN

Attachment 5 - 699 bytes
Content Type : TEXT/PLAIN

TAB 19

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Tue, 23 Mar 2004 13:13:43 -0500 (Eastern Standard Time)
Subject: Re: SNPs
To: jpevans@med.unc.edu ("Evans, Jim")
Cc: dws@email.unc.edu (Darrel Stafford),
    susswein@med.unc.edu ("Ilisa Susswein (susswein@med.unc.edu)"),
    rcnp@med.unc.edu ("Skrzynia, Cecile"),
    taoli@email.unc.edu ("'taoli@email.unc.edu'")

Hi! Jim,

The next batch (sample# 25-47) of results will come out in a couple of days. How about we have a meeting on next Tuesday afternoon? Maybe Lisa can help us to set up the meeting.

Have a nice day!

Tao

-- Begin original message --

> From: "Evans, Jim" <jpevans@med.unc.edu>
> Date: Tue, 23 Mar 2004 14:47:29 -0500
> Subject:
> To: "'taoli@email.unc.edu'" <taoli@email.unc.edu>
> Cc: Darrel Stafford <dws@email.unc.edu>,
>   "Ilisa Susswein (susswein@med.unc.edu)" <susswein@med.unc.edu>,
>   "Skrzynia, Cecile" <rcnp@med.unc.edu>
>
> Hey Tao,
>
>
>
> I'm finally back from South America! Your last email the day I left was very
> interesting. I'm especially intrigued by the 4769 G>A (3'-UTR) polymorphism.
> It is obviously very common and there is certainly precedent for 3'UTR
> variances being important.
>
>
>
> Anything new since I left?
>
>
>
> How about if we have a meeting to discuss your findings?
>
>
>
> What are good days for you? Tuesdays are often good for me.
>
>
>
> Jim
>
>
>
>
>
> James P. Evans MD, Ph.D
>
> Director of Clinical Cancer Genetics and The Program in Human Genetics
>
> Departments of Genetics and Medicine
>
> CB#7264
>
> University of North Carolina at Chapel Hill
>
> Chapel Hill, NC
>
> 27599-7264
>
> Phone: 919 843-6475
>

TAB 20

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Thu, 1 Apr 2004 16:37:23 -0500 (Eastern Standard Time)
Subject: VKOR SNPs
To: Lisa_Susswein@med.unc.edu ("Susswein, Lisa")

Hi! Lisa,

Thanks for the efforts on organizing the clinic data. This is the electronic copy of the SNP summary, thought maybe you don't need it.

Have a nice day!

Tao

There is 1 attachment in this message:

Attachment 1 - 85602 bytes
Content Type : APPLICATION/MSWORD

04/01/2004

The summary of VKOR SNPs among 47 patients

563 G>A hetero: 1/47 = 2%
: 1/47 = 2%
4769 G>A hetero: 15/47 = 32%
4769 G>A homo: 7/47 = 15%

Patient #	SNPs	Warfarin response
1		
2		
3	563 G>A hetero 4769 G>A hetero	
4	4769 G>A hetero	
5		
6		
7	4769 G>A hetero	
8		
9	4769 G>A homo	
10		
11		
12	4769 G>A homo	
13	4769 G>A homo	
14		
15	4769 G>A hetero	
16		
17	4769 G>A hetero	
18		
19		
20	4769 G>A hetero	
21	4769 G>A hetero	
22	4769 G>A hetero	
23		
24		
25	4769 G>A hetero	
26	4769 G>A homo	
27	4769 G>A hetero	
28	4769 G>A hetero	
29		
30		
31	4769 G>A homo	
32	4769 G>A hetero	
33		

34	4769 G>A hetero	
35		
36		
37	4769 G>A hetero	
38	4769 G>A homo	
39	4769 G>A homo	
40		
41	4769 G>A hetero	
42		
43		
44		
45		
46		
47		

TAB 21

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Tue, 20 Apr 2004 13:17:45 -0400 (Eastern Daylight Time)
Subject: Re: ? meeting today?
To: jpevans@med.unc.edu ("Evans, Jim"),
sussweln@med.unc.edu ("llisa Susswein (sussweln@med.unc.edu)"),
betsy_bryant@med.unc.edu (Betsy Bryant),
robb_malone@med.unc.edu (rob malone), dws@email.unc.edu (Darrel Stafford)

Hi! Jim,

Since there were no more samples to test since last meeting, probably we don't need a meeting today. By the way, I will try to make constructs with VKOR genomic DNA carrying 5'-UTR or 3'-UTR mutation, and do transient expression in mammalian cells, so that we can test if the mutation will affect the mRNA stability or transcription efficiency.

Have a nice day!

Tao

-- Begin original message --

> From: "Evans, Jim" <jpevans@med.unc.edu>
> Date: Tue, 20 Apr 2004 13:04:37 -0400
> Subject: ? meeting today?
> To: "llisa Susswein (sussweln@med.unc.edu)" <sussweln@med.unc.edu>,
> Betsy Bryant <betsy_bryant@med.unc.edu>,
> rob malone
> <robb_malone@med.unc.edu>, taoli@email.unc.edu,
> Darrel Stafford
> <dws@email.unc.edu>
>
> Hi everyone,
>
>
>
> Are we still meeting today at 4pm? Is there a reason to meet? Is there
> anything new?
>
>
>
> Jim
>
>
>
>
>
> James P. Evans MD, Ph.D
>
> Director of Clinical Cancer Genetics and The Program in Human Genetics
>
> Departments of Genetics and Medicine
>
> CB#7264
>
> University of North Carolina at Chapel Hill
>
> Chapel Hill, NC
>
> 27599-7264
>
> Phone: 919 843-6475
>
> Fax: 919 843-4682
>
> email: jpevans@med.unc.edu
>
>
>
>

-- End original message --

TAB 22

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Mon, 17 May 2004 13:54:39 -0400 (Eastern Daylight Time)
Subject: RE: males, females, and coumadin
To: jpevans@med.unc.edu ("Evans, Jim"),
    rmalone@med.unc.edu ("Malone, Robert"),
    dws@email.unc.edu (Darrel Stafford)
Cc: taoli@email.unc.edu

Hi! Jim and Rob,

How to calculate TTR from the data we have? What do you mean TTR vs. % in range INR? Does it mean we need divide the TTR by % in range INR and get a value, then look at the co-relation with the SNPs? I will ask my wife to do the analyse using SAS quickly once I get the calculated values.

Thanks!

Tao

-- Begin original message --

> From: "Evans, Jim" <jpevans@med.unc.edu>
> Date: Mon, 17 May 2004 13:31:48 -0400
> Subject: RE: males, females, and coumadin
> To: "Malone, Robert" <rmalone@med.unc.edu>, taoli@email.unc.edu,
>   Darrel Stafford <dws@email.unc.edu>
>
> Hey Tao,
>
> This thought from Rob Malone.... "Another thought is that we need to look at
> Time in Therapuetic Range(TTR) vs. % in range INR. This measure is a more
> readily accepted quality indicator in the coag community."
>
> It sounds like this would be an interesting thing to look at in relation to
> genotype....
>
>
>
> James P. Evans MD, Ph.D
> Director of Clinical Cancer Genetics and The Program in Human Genetics
> Departments of Genetics and Medicine
> CB#7264
> University of North Carolina at Chapel Hill
> Chapel Hill, NC
> 27599-7264
> Phone: 919 843-6475
> Fax: 919 843-4682
> email: jpevans@med.unc.edu
>
>
> -----Original Message-----
> From: Malone, Robert
> Sent: Monday, May 17, 2004 1:12 PM
> To: Evans, Jim
> Subject: RE: males, females, and coumadin
>
> The TTR could be calculated from the data already in the database.
>
> -----Original Message-----
> From: Evans, Jim
> Sent: Monday, May 17, 2004 12:51 PM
> To: Malone, Robert
> Subject: RE: males, females, and coumadin
>
>
>
> Hey Rob,
>
> is the TTR something that is already in your database, or a statistic that
> could be derived from the raw data already there?
>
> Darrel is submitting a grant, and perhaps there would be some time in it (if
> funded) for someone to work on getting missing data into the database.
>

> jim
>
> -----Original Message-----
> From: Malone, Robert
> To: Evans, Jim
> Sent: 5/14/04 6:15 PM
> Subject: RE: males, females, and coumadin
>
> Yes. There are a few mssing entries, especially for patients that have
> been followed longer. Since the database was developed on the fly, some
> records may be missing total weekly doses.
>
> Another thought is that we need to look at Time in Therapuetic Range
> (TTR) vs. % in range INR. This measure is a more readily accepted
> quality indicator in the coag community.
>
> -----Original Message-----
> From: Evans, Jim
> To: Malone, Robert; ''taoli@email.unc.edu' '
> Cc: 'llisa Susswein (susswein@med.unc.edu) '; 'rob malone '; 'Betsy
> Bryant '; 'Darrel Stafford '
> Sent: 5/14/04 11:48 AM
> Subject: RE: males, females, and coumadin
>
> Hey Rob,
>
> Sounds good. What are you referring to with respect to the missing data?
> Do you mean in the database as a whole?
>
> Jim
>
> James P. Evans MD, Ph.D
> Director of Clinical Cancer Genetics and The Program in Human Genetics
> Departments of Genetics and Medicine
> CB#7264
> University of North Carolina at Chapel Hill
> Chapel Hill, NC
> 27599-7264
> Phone: 919 843-6475
> Fax: 919 843-4682
> email: jpevans@med.unc.edu
>
>
> -----Original Message-----
> From: Malone, Robert
> Sent: Friday, May 14, 2004 11:16 AM
> To: Evans, Jim; ''taoli@email.unc.edu' '
> Cc: 'llisa Susswein (susswein@med.unc.edu) '; 'rob malone '; 'Betsy
> Bryant '; 'Darrel Stafford '
> Subject: RE: males, females, and coumadin
>
> The comparrison would be no problem. I'll look when we get back.
>
> If you think it would be valuable, it may be worth having someone start
> to enter missing data such as warfarin dose, INR, etc.
>
> -----Original Message-----
> From: Evans, Jim
> To: 'taoli@email.unc.edu'
> Cc: llisa Susswein (susswein@med.unc.edu); rob malone; Betsy Bryant;
> Darrel Stafford
> Sent: 5/14/04 8:31 AM
> Subject: RE: males, females, and coumadin
>
> Thanks, Tao.
>
> This male-female difference really does look intriguing. Although the
> numbers are small they appear amazingly convincing. I'm sure it could
> fall
> apart with more people, but I think we should definitely follow it up.
>
> Rob and Besty, how hard would it be to simply compare average coumadin
> dose
> for all males vs. all females in the database?
>

> Jim
>
> PS. Rob and Betsy, don't worry about writing up a "blurb" about the
> clinic.
> I did that and will run it by you to be sure you approve.
>
> Jim
>
>
>
> James P. Evans MD, Ph.D
> Director of Clinical Cancer Genetics and The Program in Human Genetics
> Departments of Genetics and Medicine
> CB#7264
> University of North Carolina at Chapel Hill
> Chapel Hill, NC
> 27599-7264
> Phone: 919 843-6475
> Fax: 919 843-4682
> email: jpevans@med.unc.edu
>
>
> -----Original Message-----
> From: taoli@email.unc.edu [mailto:taoli@email.unc.edu]
> Sent: Friday, May 14, 2004 1:51 AM
> To: Evans, Jim
> Cc: Darrel Stafford
> Subject: Re: males, females, and coumadin
>
> Hi! Jim,
>
> Based on current data, the difference of the average dose between male
> and female is very apparent. However, I think at this point it's hard
> to say if the statistical analyzing can be genelized because we have
> less than 50 patients.
>
> Here I attach the slides I showed you that day. If you need the
> original SAS files, I can ask my wife to forward it to you, and she
> will explain the result.
>
> Have a nice day!
>
> Tao
>
> Quoting "Evans, Jim" <jpevans@med.unc.edu>:
>
> > Hey Darrel and Tao,
> >
> >
> >
> > Interesting news on the male-female-coumadin front. I asked Stephan
> > Moll,
> > who is a clinical coag guru and he has never heard of a sex
> > difference in
> > coumadin response.
> >
> >
> >
> > It seems bizarre to me that a real association would not have been
> > discovered, but maybe your results are real. Tao, can you send me
> > the
> > statistics that you showed us the other day? What is the magnitude of
> > the
> > difference? How likely is this that your finding of a difference in
> > coumadin
> > dosage by sex was chance?
> >
> >
> >
> > Jim
> >
> >
> >
> >
> >
> >

TAB 23

06/07/2004

UNCStudyNo	563G>A het	4501C>T het	4769G>A het	4769G>A homo	Gender	Race	Number INI
1	No	No	No	No	Male	African	32
2	No	No	No	No	Male	Caucasian	9
3	Yes	Yes	Yes	No	Female	African	72
4	No	No	Yes	No	Male	African	19
5	No	No	No	No	Male	Caucasian	16
6	No	No	No	No	Male	Caucasian	19
7	No	No	Yes	No	Male	Caucasian	34
8	No	No	No	No	Male	Caucasian	37
9	No	No	No	Yes	Male	Caucasian	16
10	No	No	No	No	Female	African	25
11	No	No	No	No	Male	Caucasian	42
12	No	No	No	Yes	Male	Caucasian	10
13	No	No	No	Yes	Female	Caucasian	26
14	No	No	No	No	Male	Caucasian	34
15	No	No	Yes	No	Female	Caucasian	19
16	No	No	No	No	Male	Caucasian	5
17	No	No	Yes	No	Male	Caucasian	15
18	No	No	No	No	Male	African	8
19	No	No	No	No	Male	Caucasian	14
20	No	No	Yes	No	Female	Caucasian	19
21	No	No	Yes	No	Female	Caucasian	18
22	No	No	Yes	No	Male	Caucasian	28
23	No	No	No	No	Male	Caucasian	24
24	No	No	No	No	Female	African	49
25	No	No	Yes	No	Male	Caucasian	10
26	No	No	No	Yes	Male	Caucasian	10
27	No	No	Yes	No	Female	African	8
28	No	No	Yes	No	Female	Caucasian	56
29	No	No	No	No	Female	Caucasian	9
30	No	No	No	No	Male	Caucasian	23
31	No	No	No	Yes	Male	African	39
32	No	No	Yes	No	Male	Caucasian	46
33	No	No	No	No	Female	Caucasian	41
34	No	No	Yes	No	Male	Caucasian	16
35	No	No	No	No	Female	Caucasian	35
36	No	No	No	No	Male	African	18
37	No	No	Yes	No	Male	African	26
38	No	No	No	Yes	Male	African	17
39	No	No	No	Yes	Female	Caucasian	15
40	No	No	No	No	Male	Caucasian	26
41	No	No	Yes	No	Male	Caucasian	20
42	No	No	No	No	Male	Caucasian	50
43	No	No	No	No	Male	Caucasian	33
44	No	No	No	No	Female	Caucasian	18
45	No	No	No	No	Female	Caucasian	12
46	No	No	No	No	Female	Caucasian	15
47	No	No	No	No	Male	Caucasian	7

AVG INR	Min INR	Max INR	SD INR	VarianceINR	umber Dos	AVG Dose	MinDose	Max Dose
2.45625	1.1	6.3	1.1103435	1.2328629	28	43.303571	7.5	55
3.0111111	1.8	4.9	1.0397649	1.0811111	10	16.9	14	24.5
2.8333333	0.9	8	1.8230140	3.3233802	72	101.66666	0	150
2.8842105	1.2	6.2	1.3586198	1.8458479	18	24.166666	14	30
2.175	1.1	4	0.7261772	0.5273333	16	68.125	27.5	80
2.2	1.4	4.2	0.7071067	0.5	18	37.361111	35	40
2.5911764	1.5	4.2	0.5523326	0.3050713	33	33.106060	30	35
3.3081081	1.2	6.4	1.0850955	1.1774324	38	49.631578	31	63
2.3625	1.4	4.7	0.8913472	0.7944999	13	65.961538	52.5	77.5
2.588	1.2	4.7	0.9143850	0.8361000	25	24.22	19.5	28.5
2.4571428	1.4	4.4	0.7421599	0.5508013	42	16.309523	12.5	21.25
2.62	1.3	3.7	0.7284687	0.5306666	10	33.5	25	37.5
2.5769230	1	4.8	1.1378251	1.2946461	26	57.211538	40	60
2.9235294	1.2	6.1	1.0865733	1.1806417	33	18.181818	12.5	26.25
2.0684210	0.9	3.7	0.8583269	0.7367251	17	63.308823	28.75	95
2.36	1.3	5.2	1.6471186	2.713	2	24.125	22	26.25
2.2066666	1.5	3.4	0.6273147	0.3935238	15	48	35	52.5
2.25	1.5	4.2	0.9133924	0.8342857	2	35	35	35
3.1785714	1.7	5.4	1.1636669	1.3541208	13	38.461538	34	42
2.2842105	1.4	4.3	0.6865883	0.4714035	19	22.763157	0	27
2.7722222	1.3	7.6	1.4756077	2.1774183	18	50	0	62.5
2.2678571	1.6	3.6	0.4338062	0.1881878	27	24.768518	22.5	27.5
2.0875	1.1	3.5	0.5620710	0.3159239	23	24.239130	15	27.5
1.9326530	0.8	5.7	1.3882524	1.9272448	48	111.45833	0	150
2.15	1.5	3	0.4624812	0.2138888	9	40.277777	35	42.5
2.48	1.8	3.2	0.4848825	0.2351111	9	41.666666	25	45
2.1375	1.5	2.6	0.4373213	0.19125	6	35.416666	35	37.5
2.7375	1	8	1.3186166	1.7387499	48	56.770833	0	75
2.9444444	1.6	5	1.1159201	1.2452777	9	31.111111	28	31.5
2.4956521	1.6	3.2	0.5049556	0.2549802	22	13.806818	10	15
2.5128205	1.4	5.3	0.7664612	0.5874628	38	46.447368	40	57.5
2.2956521	1.2	4.4	0.5617261	0.3155362	45	31.944444	0	37.5
3.1731707	1.5	5.5	1.0495295	1.1015121	37	62.905405	47.5	80
2.7375	1.4	6.1	1.2252210	1.5011666	5	29	22.5	35
2.4257142	1.5	3.4	0.5054659	0.2554957	34	33.970588	27.5	35
2.3444444	1.7	4	0.6335912	0.4014379	13	8.6153846	7	10
3.0923076	2	5.9	0.8939454	0.7991384	24	58.645833	52.5	70
2.9	1.2	5.7	1.2489995	1.5599999	15	40.333333	32.5	55
2.1866666	1.4	4.4	0.7170043	0.5140952	15	64.666666	52.5	75
1.8	1	4.2	0.6584831	0.4336	26	35.769230	0	45
2.71	1.6	5.7	1.1368932	1.2925263	18	16.527777	0	35
2.718	1.3	5	0.8532506	0.7280367	49	25.020408	17.5	35
3.2030303	2.1	4.4	0.5870928	0.3446780	33	24.056060	22.5	28.75
3.3055555	1.4	8	1.7396660	3.0264379	13	38.576923	0	70
2.075	1	3.1	0.6397797	0.4093181	10	46.25	25	60
2.72	1.3	7.5	1.5621413	2.4402857	14	27.857142	17.5	47.5
2.5571428	1.6	3.6	0.6827814	0.4661904	6	41.75	40.5	42

SD_Dose	arianceDos	DoseCh	DoseCha	DoseChak	DoseCha	DoseChance	DoseC	Indic1
9.2809435	86.135912	27	43.240740	7.5	55	9.0089382	81.160968	Atrial
3.5496478	12.6	9	16.111111	14	20.5	2.4593924	6.0486111	Prophylaxi
23.092740	533.27464	67	99.552238	0	150	28.062178	787.48586	Prosthetic
5.2131056	27.176470	19	22.368421	0	28	7.2107984	51.995614	Prosthetic
15.152007	229.58333	16	69.21875	37.5	80	13.125	172.26562	Treatment
1.8133942	3.2883986	18	37.638888	35	40	1.8133942	3.2883986	Atrial
2.3410653	5.4805871	32	32.890625	30	35	2.3852178	5.6892641	Atrial
7.6984415	59.266002	38	49.973684	31	63	7.7581233	60.188477	Prosthetic
7.8088132	60.977564	13	66.730769	52.5	77.5	8.4400221	71.233974	Prosthetic
3.0757112	9.46	25	24.48	19.5	28.5	3.0838287	9.51	Prosthetic
1.9512421	3.8073461	42	16.517857	12.5	21.25	1.8833293	3.5469294	Prophylaxi
3.3747427	11.388888	10	33.25	27.5	37.5	2.8987545	8.4027777	Atrial
5.5824243	31.163461	26	57.788461	45	60	4.2027921	17.663461	Atrial
3.0633115	9.3838778	33	18.484848	12.5	26.25	3.0097104	9.0583570	Prophylaxi
17.449375	304.48069	18	64.583333	35	90	14.608871	213.41911	Treatment
3.0052038	9.03125	2	25.625	25	26.25	0.8838834	0.78125	Recurrent
5.7631836	33.214285	15	49.5	40	52.5	4.2468475	18.035714	Prophylaxi
0	0	2	37.5	35	40	3.5355339	12.5	Atrial
3.1784530	10.102564	13	37.538461	32	42	3.6655009	13.435897	Prosthetic
6.0881679	37.065789	19	23.184210	0	27	5.9703213	35.644736	Atrial
14.271526	203.67647	16	54.0625	40	62.5	5.7644745	33.229166	Treatment
1.6997255	2.8890669	27	24.953703	23.75	27.5	1.7153675	2.9424857	Atrial
2.9613785	8.7697628	23	23.478260	0	27.5	5.9227570	35.079051	Prophylaxi
27.809349	773.35992	48	116.35416	70	155	20.903516	436.95700	Prophylaxi
2.3199018	5.3819444	9	41.111111	40	42.5	1.3176156	1.7361111	Treatment
6.7314560	45.3125	9	44.166666	40	45	1.7677669	3.125	Treatment
1.0206207	1.0416666	5	35.5	35	37.5	1.1180339	1.25	Prophylaxi
16.142634	260.58466	47	60.297872	42.5	75	9.9393047	98.789777	Prophylaxi
1.1666666	1.3611111	9	30.944444	28	31.5	1.2104865	1.4652777	Prophylaxi
1.4677724	2.1543560	22	13.863636	10	15	1.4895015	2.2186147	Atrial
4.5999257	21.159317	38	46.710526	40	57.5	4.5061993	20.305832	Femoral
6.0978966	37.184343	43	32.558139	0	37.5	5.3588037	28.716777	Atrial
10.712685	114.76163	35	63.5	47.5	80	10.257708	105.22058	Prosthetic
5.7554322	33.125	4	26.25	17.5	35	7.2168783	52.083333	Prophylaxi
2.2290818	4.9688057	28	34.107142	25	35	2.3779743	5.6547619	Atrial
1.2102659	1.4647435	13	8.7307692	7	10	1.1108439	1.2339743	Pulmonary
7.5892933	57.597373	24	57.604166	45	70	7.6782855	58.956068	Atrial
7.7267315	59.702380	13	38.846153	32.5	55	7.9461327	63.141025	Treatment
6.7391888	45.416666	15	66.5	55	75	5.4935026	30.178571	Prophylaxi
11.592437	134.38461	26	38.942307	27.5	45	5.1562135	26.586538	Prophylaxi
7.3708931	54.330065	16	15.46875	0	27.5	6.1215976	37.473958	Atrial
5.1231797	26.246970	47	24.925531	17.5	35	4.9549368	24.551399	Atrial
1.5364710	2.3607433	32	23.945312	22.5	28.75	1.5262942	2.3295740	Prosthetic
18.849012	355.28525	12	37.791666	25	70	13.538595	183.29356	Prosthetic
15.011569	225.34722	12	50.416666	30	60	11.910334	141.85606	Treatment
9.7496125	95.054945	13	28.076923	17.5	45	8.7888332	77.243589	Treatment
0.6123724	0.375	6	41.5	39	42	1.2247448	1.5	Treatment

Indic2	Indic3	Ind_Meno	Goal INR	Diabetes	Renal	Dialysis	HTN	CABG
Recurrent	Not	He has a	2 - 3	Not	No	Not	Not	Not
Not	Not	Dissection	2 - 3	Not	Not	Not	Not	Not
Not	Not	Mechanic	2 - 3	No	No	No	Yes	No
Femoral	Prophylaxi	Fem pop	2 - 3	No	No	No	Yes	Yes
Not	Not	DVT dx	2 - 3	No	No	No	No	No
Not	Not	No	2 - 3	Yes	No	No	Yes	No
Not	Not	No	2 - 3	No	No	No	Yes	No
Prophylaxi	Not	Lupus	2.5 - 3.5	No	Yes	No	Yes	No
Not	Not	AVR	2 - 3	Yes	Not	No	Yes	Yes
Not	Not	Mechanic	2 - 3	Not	Not	Not	Not	Not
MI Acute	Not	CVA	2 - 3	Not	Yes	Not	Not	Yes
Not	Not	Atrial	2 - 3	Not	Not	Not	Not	Not
Not	Not	No	2 - 3	Not	No	Not	Yes	Not
Not	Not	antiphosp	2.5 - 3.5	No	Not	Not	No	Not
Not	Not	Antiphosp	2 - 3	Yes	No	No	Yes	Yes
Prophylaxi	Not	DVT/PE,	2 - 3	No	No	No	Yes	No
Not	Not	Rt	2 - 3	Not	Not	Not	Not	Not
Not	Not	No	2 - 3	Not	Not	Not	Not	Not
Not	Not		2 - 3	No	No	No	No	No
Not	Not	No	2 - 3	No	No	No	Yes	No
Not	Not	R and L	2 - 3	No	No	No	Yes	No
Not	Not	Failed	2 - 3	No	No	No	No	No
Not	Not	DVT 6/02.	1.5 - 2	Not	No	Not	Not	Not
Not	Not	H/O DVT	1.5 - 2	Yes	No	No	Yes	No
Not	Not	dvt	2 - 3	Not	Not	Not	Not	Not
Not	Not	No	2 - 3	No	No	No	No	No
Not	Not	PVD, CVA	2 - 3	Not	Not	Not	Not	Not
Not	Not	Factor V	2 - 3	No	No	No	No	No
Not	Not	PE x 2	1.5 - 2.5	Not	Not	Not	Not	Not
Not	Not	No	2 - 3	No	Not	Not	Yes	Not
Not	Not	Aortofemo	2.5 - 3.5	No	No	No	No	No
Not	Not	No	2 - 3	No	No	No	Yes	Yes
Not	Not	st jude	2.5 - 3.5	No	No	No	Yes	Yes
Not	Not	PE/DVT	2 - 3	Not	Not	Not	Not	Not
Not	Not	No	2 - 3	No	No	No	Yes	No
Prophylaxi	Not	DVT x 2	1.5 - 2	Not	No	Not	Yes	Not
Not	Not	No	2 - 3	No	No	No	Yes	No
Not	Not	DVT/PE;	2 - 3	No	Yes	Not	Yes	Yes
Recurrent	Not	Active tx	2 - 3	Not	Not	Not	Not	Not
Not	Not	DVT -	1.5 - 2	Not	Yes	Not	Not	Not
Not	Not	No	2 - 3	No	No	No	Yes	No
MI Acute	Not	(L) Atrial	2 - 3	No	Yes	No	Yes	No
Atrial	Prophylaxi	Also DVT	2.5 - 3.5	No	Yes	No	Yes	No
Not	Not	Previous	2.5 - 3.5	Not	Not	Not	Yes	Not
Not	Not	Bilateral	2 - 3	Not	Not	Not	Not	Not
Atrial	Not	LLE DVT	2 - 3	Not	Not	Not	Not	Not
Not	Not	DVT in	2 - 3	Not	Not	Not	Not	Not

MI	CHF	CVA/TIA	Malignancy	GIBleed	Hepatic	moking Stat	k Year His	EtOH Statu
Not	Not	Not	Not	Not	No	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
Yes	No	No	No	No	No	Current		Occasiona
Yes	No	Yes	No	No	No	Current		Previous
No	No	No	No	No	No	Never		Not
No	No	No	No	No	No	Not		Not
No	No	Yes	No	No	No	Never		Occasiona
No	No	No	No	No	No	Not		Not
Not	Not	Not	Not	Not	Not	Current		Not
Not	Yes	Not	Not	Not	No	Distant		Not
Yes	Yes	Yes	Not	Not	Not	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
Not	Not	Not	Yes	Not	Not	Not		Not
Not	Not	Not	Not	Not	Not	Current		Not
Yes	No	No	No	No	No	Not		Not
No	No	Yes	No	Yes	Not	Current		Abusive
Not	Not	Not	Not	Not	Not	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
No	No	No	No	No	No	Not		Not
No	No	No	Not	No	No	Not		Not
No	No	No	No	No	No	Current		Not
No	Yes	No	No	No	No	Distant		Previous
Not	Not	Not	Not	Not	No	Not		Not
No	No	No	No	No	No	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
No	No	No	No	No	No	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
No	Yes	No	No	No	No	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
Not	Yes	Not	Not	Not	Yes	Not		Not
No	No	No	No	No	No	Current		Not
Yes	Yes	No	Yes	No	No	Never		Occasiona
No	No	Yes	No	No	No	Current	50	Occasiona
Not	Not	Not	Not	Not	Not	Not		Not
No	No	Yes	No	No	No	Distant		Occasiona
Not	Not	Yes	Not	Not	Not	Never		Occasiona
No	Yes	No	No	No	No	Distant		Abusive
Yes	No	No	No	No	No	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
No	No	No	No	No	No	Not		Not
Yes	No	No	No	No	No	Never		Occasiona
No	Yes	Yes	No	No	Yes	Not		Not
Not	Not	Not	Not	Not	Yes	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not
Not	Not	Not	Not	Not	Not	Not		Not

TAB 24

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Mon, 14 Jun 2004 16:32:24 -0400 (Eastern Daylight Time)
Subject: Re: VKOR study
To: jpevans@med.unc.edu ("Evans, Jim"), dws@email.unc.edu (Darrel Stafford),
susswein@med.unc.edu ("llisa Susswein (sussweln@med.unc.edu)"),
rcnp@med.unc.edu ("Skrzynia, Cecile")

Hi! Jim and Lisa,

Thank you for making the clinic works again! I will do some in vitro studies and bioinformatics research at this period.

Have a good one!

Tao

-- Begin original message --

> From: "Evans, Jim" <jpevans@med.unc.edu>
> Date: Mon, 14 Jun 2004 16:09:58 -0400
> Subject: VKOR study
> To: Darrel Stafford <dws@email.unc.edu>, taoli@email.unc.edu,
> "llisa Susswein (sussweln@med.unc.edu)" <sussweln@med.unc.edu>,
> "Skrzynia, Cecile" <rcnp@med.unc.edu>
>
> Hey Darrel and Tao,
>
>
>
> Lisa let me know that we just got IRB approval to start calling the folks
> whom we have been unable to catch in coag clinic. That was the hold-up, but
> now that we have approval more samples should be coming soon.
>
>
>
> I also have calls in to the cardiology clinic director to see if we can
> start assaulting (er...I mean asking) their patients for permission to be in
> the study.
>
>
>
> Jim
>
>
>
>
>
>
>
> James P. Evans MD, Ph.D
>
> Director of Clinical Cancer Genetics and The Program in Human Genetics
>
> Departments of Genetics and Medicine
>
> CB#7264
>
> University of North Carolina at Chapel Hill
>
> Chapel Hill, NC
>
> 27599-7264
>
> Phone: 919 843-6475
>
> Fax: 919 843-4682
>
> email: jpevans@med.unc.edu
>
>
>
>

-- End original message --

TAB 25

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Thu, 24 Jun 2004 10:36:19 -0400 (Eastern Daylight Time)
Subject: Re: coumadin schedule
To: Lisa_Gatti@med.unc.edu

Than you, Lisa. See you next Monday. ---Tao

-- Begin original message --

> From: "Lisa R. Gatti" <lgatti@med.unc.edu>
> Date: Thu, 24 Jun 2004 09:13:47 -0400
> Subject: coumadin schedule
> To: Gini Bright <vbright@EMAIL.UNC.EDU>
> CC: susswein@med.unc.edu, taoli@EMAIL.UNC.EDU
> Reply-To: Lisa_Gatti@med.unc.edu
>
>
> Gini/Lisa/Tao,
>
> It seems (as Gini has also reported) that there are no morning coumadin
> patients today (Thursday). There are some scheduled for this afternoon,
> but the staff requested that I do not recruit on THurs. afternoons- as
> they hold extra clinics in that space. And, there are NO coumadin
> patients scheduled for Friday (tomorrow) at all. Maybe Betsy is going
> out of town?? So, unfortunately, I won't be getting any samples untill
> the beginning of next week. It seems that clinics have different issues
> in the summer (vacations) than in the winter (bad weather)!!
> Hopefully next week things will really pick up!
> Gini, just email me if you talk to anyone for Mon/Tues.
> Thanks!
> Lisa G.
>

-- End original message --

TAB 26

Applied Biosystems Store: Order Summary

Summary

For ordering help please call 1-800-327-3002 or email us.

Contact Information

Name Tao Li

Grant Number 5-51536

Phone Number 919-962-2267

Department Biology

Shipping and Billing Information

Shipping Information

University of North Carolina
Dept of Biology
Attn: Dr Tao Li
438 Wilson Hall
CHAPEL HILL, NC 27599
United States

Billing Information

University of North Carolina
Department of Biology
Attn: Accounting
Coker Hall Campus Box 3280
CHAPEL HILL, NC 27599
United States

Sales Order Number:185185780

Date Submitted:06/30/2004

Shipping Method: Federal Express - Priority Overnight

Payment Method Purchase Order

Purchase Order #: K-371538

Special Instructions: I've canceled my previous order #185185507 and replace it with this one.

Product Number	Qty	Your Average Price	Your Extended Price
1 Custom TaqMan® SNP Genotyping Assays, Medium-Scale, human 40X Concentration 3,000 (5uL) reactions [Details]			
4332072	1	USD 551.00	USD 551.00

File: VKOR3UTR.txt [12 days left before this file and line item are deleted]

Estimated Ship Date: 07/22/2004

Requested Ship Date:: 06/30/2004

Subtotal:	USD 551.00
Freight:	*
Tax:	USD 38.58
Total:	USD 589.58

*Freight charges are added at time of shipment for instrument or standing orders or newly registered customers. Consumable products with subtotals over $50,000 USD are shipped free of charge.

Custom Products may not be cancelled. Please refer to our Order Cancellation and Return Policy.

† Assays-by-Design products take
upto 16 business days for individual assays between 1-3000
upto 22 business days for individual assays between 3001 - 6000

Returns will not be accepted without prior authorization from Applied Biosystems. For return authorization, contact Customer Service. Merchandise sent in error by Applied Biosystems are returnable for refund, exchange or credit. Merchandise ordered in error by the customer are not returnable so please be sure to verify all items before placing an order.

Applied Biosystems Store: Order Summary

Summary

For ordering help please call 1-800-327-3002 or email us.

Contact Information

Name Tao Li

Grant Number 5-51536

Phone Number 919-962-2267

Department Biology

Shipping and Billing Information

Shipping Information

UNC-CH
Biology
Tao Li
438 Wilson Hall
Chapel Hill, NC 27599
United States

Billing Information

UNC-CH
Biology
Darrel Stafford
442 Wilson Hall
PO BOX: 3280
Chapel Hill, NC 27599
United States

Sales Order Number:185185507

Shipping Method: Federal Express - Priority Overnight

Date Submitted:06/29/2004

Payment Method Purchase Order

Purchase Order #: K-371538

Product Number	Qty	Your Average Price	Your Extended Price
1: Custom TaqMan® SNP Genotyping Assays, Medium-Scale, human 40X Concentration 3,000 (5uL) reactions [Details]			
4332072	1	USD 580.00	USD 580.00

File: VKORSNP.txt [12 days left before this file and line item are deleted]

Estimated Ship Date: 06/29/2004

Requested Ship Date:: 06/30/2004

Subtotal:	USD 580.00
Freight:	+
Tax:	USD 0.00
Total:	USD 580.00

*Freight charges are added at time of shipment for instrument or standing orders or newly registered customers. Consumable products with subtotals over $50,000 USD are shipped free of charge.

Custom Products may not be cancelled. Please refer to our Order Cancellation and Return Policy.

† Assays-by-Design products take
upto 16 business days for individual assays between 1-3000
upto 22 business days for individual assays between 3001 - 6000

Returns will not be accepted without prior authorization from Applied Biosystems. For return authorization, contact Customer Service. Merchandise sent in error by Applied Biosystems are returnable for refund, exchange or credit. Merchandise ordered in error by the customer are not returnable so please be sure to verify all items before placing an order.

Printed by: Tao Li

From: orders@appliedbiosystems.com
Date: Wed, 30 Jun 2004 09:34:16 -0700 (PDT)
Subject: Applied Biosystems Store Sales Order#: 185185507
To: taoli@email.unc.edu, orders@appliedbiosystems.com
Reply-To: orders@appliedbiosystems.com

Sales Order #: 185185507
Purchase Order #: K-371538
Date Submitted: 06/29/2004

Dear Tao Li,

Thank you for ordering from the Applied Biosystems Store! Your order information appears below. If you have any questions, please contact Customer Service at 1-800-327-3002 or customer.service@appliedbiosystems.com and reference your sales order number 185185507.

We will notify you by e-mail when your order ships. You may check the status of your order online at: http://store.appliedbiosystems.com and clicking the order inquiry link.

-- Your order contains Assays-by-Design product. Important information concerning your order is noted below following the "Sales Order Details."

Sales Order Details:

Shipping Address:
University of North Carolina
Dept of Biology
Attn: Dr Tao Li
438 Wilson Hall

CHAPEL HILL, NC 27599, United States

Telephone:

Billing address:
University of North Carolina
Department of Biology
Attn: Accounting
Coker Hall Campus Box 3280

CHAPEL HILL, NC 27599, United States

Sales Order #: 185185507
Date Submitted: 06/29/2004
Basket Name: Regular:My Basket
Purchase Order#: K-371538
Payment Method: PO
Shipping Method: Federal Express - Priority Overnight
Special Instructions:

Product Name / Product Number	Qty	Est. Ship Date	Your Ext. Price
Custom TaqMan SNP Genotyping Assays, Medium-Scale, human 40X Concentration 3,000 (SuL) reactions 4332072 File: VKORSNP.txt	1--	07/22/2004	USD 551.00

Item Total- USD	551.00
Freight- USD	*
Tax- USD	38.58
Order Total- USD	589.58

* Applicable freight charges will be added at time of shipment. Applied Biosystems will pay your freight f consumable products if their subtotal is greater than $50,000 USD.

-- Important Information

Before an Assays-by-Design is manufactured it is designed using our proprietary design algorithms using the submitted sequence. All assays are designed for use with Universal Master Mix and the universal cycling conditions. For certain DNA target sequences, such as repeats, ambiguous bases and/or

TAB 27

Applied Biosystems Store: Order Summary

Summary

For ordering help please call 1-800-327-3002 or email us.

Contact Information

Name Tao Li

Grant Number 5-51536

Shipping and Billing Information

Shipping Information

University of North Carolina
Dept of Biology
Attn: Dr Tao Li
438 Wilson Hall
CHAPEL HILL, NC 27599
United States

Sales Order Number:185187448

Shipping Method: Federal Express - Priority Standard

Phone Number 919-962-2267

Department Biology

Billing Information

University of North Carolina
Department of Biology
Attn: Accounting
Coker Hall Campus Box 3280
CHAPEL HILL, NC 27599
United States

Date Submitted:07/07/2004

Payment Method Purchase Order

Purchase Order #: K-379093

	Product Number	Qty	Your Average Price	Your Extended Price
1.	Custom TaqMan® SNP Genotyping Assays, Medium-Scale, human 40X Concentration 3,000 (5uL) reactions [Details]	1	USD 551.00	USD 551.00
	4332072			
	File: VKORSUTR.txt [12 days left before this file and line item are deleted]			Requested Ship Date:: 07/07/2004
	Estimated Ship Date: 07/29/2004			
2.	Custom TaqMan® SNP Genotyping Assays, Medium-Scale, human 40X Concentration 3,000 (5uL) reactions [Details]	1	USD 551.00	USD 551.00
	4332072			
	File: VKOR2581.txt [12 days left before this file and line item are deleted]			Requested Ship Date:: 07/07/2004
	Estimated Ship Date: 07/29/2004			
3.	Custom TaqMan® SNP Genotyping Assays, Medium-Scale, human 40X Concentration 3,000 (5uL) reactions [Details]	1	USD 551.00	USD 551.00
	4332072			
	File: VKOR3294.txt [12 days left before this file and line item are deleted]			Requested Ship Date:: 07/07/2004
	Estimated Ship Date: 07/29/2004			

P/N: 4331349, 4332072, 4332073, 4332077, 4332075, 4332076

Overview

The assay reagents for Single Nucleotide Polymorphism (SNP) genotyping from the Assays-by-Design℠ service consist of a 40X or 80X mix of unlabeled PCR primers and TaqMan® MGB probes (FAM™ and VIC® dye-labeled). These assays are designed for the genotyping of specific SNPs. Each assay enables scoring of both alleles in a single well. All assays are optimized to work with TaqMan® Universal PCR Master Mix, No AmpErase® UNG (P/N 4324018) or TaqMan® Universal PCR Master Mix (P/N 4304437) and with genomic DNA. These products utilize the universal thermal cycling parameters described below in Table 2.

Procedure

To prepare the reaction components for a single 5µL reaction (384-well plate) or a single 25µL reaction (96-well plate) refer to the tables below.

Table 1a. Allelic Discrimination PCR Reaction 40X mix (part numbers 4331349, 4332072, 4332077 and 4332075)

Reaction Component	Volume/Well *(5µL volume reaction [1])*	Volume/Well *(25µL volume reaction [1])*	Final Concentration
TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2X)	2.5	12.5	1X
40X Assay Mix	0.125	0.625	1X
Genomic DNA diluted in dH_2O [2]	2.375	11.875	--
Total	5	25	

1. If different volumes are used, amounts should be adjusted accordingly.
2. 1-20 ng of genomic DNA.

Table 1b. Allelic Discrimination PCR Reaction 80X mix (part numbers 4332073 and 4332076)

Reaction Component	Volume/Well *(5µL volume reaction [3])*	Volume/Well *(25µL volume reaction [3])*	Final Concentration
TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2X)	2.5	12.5	1X
80X Assay Mix	0.0625	0.3125	1X
Genomic DNA diluted in dH_2O [4]	2.4275	12.1875	--
Total	5	25	

3. If different volumes are used, amounts should be adjusted accordingly.
4. 1-20 ng of genomic DNA.

MATERIAL SAFETY DATA SHEET

SECTION 1 CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(650) 570-6667 (USA)
01925-825650 (UK)

24 HOUR EMERGENCY RESPONSE NUMBER:
1-800-424-9300 (NORTH AMERICA)
1-703-527-3887 (INTERNATIONAL)

SUBSTANCE: CUSTOM OLIGONUCLEOTIDES (MGB)

TRADE NAMES/SYNONYMS:
MSDS P/N 4332728; EHS1000258; P/N 4324035; P/N 4331181; P/N 4331182; P/N 4331183; P/N 4331348; P/N 4331349; P/N 4332072; P/N 4332073; P/N 4332075; P/N 4332076; P/N 4332077; P/N 4332078; P/N 4332079; P/N 4337216; P/N 4337217; P/N 4337218; P/N 4337219; P/N 4337220; P/N 4337221; P/N 4337222; P/N 4337223; P/N 4337224; MGB SNP SET; MGB GENEX SET; ASSAYS-ON-DEMAND(TM) SNP GENOTYPING PRODUCTS; ASSAYS-ON-DEMAND(TM) GENE EXPRESSION PRODUCTS; ASSAYS-BY-DESIGN(SM)
SERVICE; 00227420

PRODUCT USE: For Research Use Only. Not for use in diagnostic procedures.

CREATION DATE: Nov 08 2001
REVISION DATE: Feb 05 2003

SECTION 2 COMPOSITION, INFORMATION ON INGREDIENTS

COMPONENT: OTHER NONHAZARDOUS COMPONENTS
CAS NUMBER: Not assigned.
PERCENTAGE: >75

COMPONENT: FORMAMIDE, RECRYSTALLIZED
CAS NUMBER: 75-12-7
PERCENTAGE: 1-20

COMPONENT: ETHYLENEDIAMINETETRAACETIC ACID
CAS NUMBER: 60-00-4
PERCENTAGE: <1

COMPONENT: OLIGONUCLEOTIDE PRIMERS/PROBES
CAS NUMBER: Not assigned.
PERCENTAGE: <0.1

FIRE FIGHTING: Move container from fire area if it can be done without risk. Avoid inhalation of material or combustion by-products. Stay upwind and keep out of low areas.

FLASH POINT: aqueous solution

SECTION 6 ACCIDENTAL RELEASE MEASURES

OCCUPATIONAL RELEASE:
Stop leak if possible without personal risk. Small spills: Absorb with sand or other non-combustible material. Collect spilled material in appropriate container for disposal. Notify Local Emergency Planning Committee and State Emergency Response Commission for release greater than or equal to RQ (U.S. SARA Section 304). If release occurs in the U.S. and is reportable under CERCLA Section 103, notify the National Response Center at (800)424-8802 (USA) or (202)426-2675 (USA).

SECTION 7 HANDLING AND STORAGE

STORAGE: Store and handle in accordance with all current regulations and standards. See original container for storage recommendations. Keep separated from incompatible substances.

SECTION 8 EXPOSURE CONTROLS, PERSONAL PROTECTION

EXPOSURE LIMITS:
FORMAMIDE:
20 ppm (30 mg/m3) OSHA TWA (vacated by 58 FR 35338, June 30, 1993)
30 ppm (45 mg/m3) OSHA STEL (vacated by 58 FR 35338, June 30, 1993)
10 ppm ACGIH TWA (skin)
10 ppm (15 mg/m3) NIOSH recommended TWA 10 hour(s) (skin)

VENTILATION: Provide local exhaust ventilation system. Ensure compliance with applicable exposure limits.

EYE PROTECTION: Wear splash resistant safety goggles with a faceshield. Provide an emergency eye wash fountain and quick drench shower in the immediate work area.

CLOTHING: Wear appropriate chemical resistant clothing.

GLOVES: Wear appropriate chemical resistant gloves.

RESPIRATOR: Under conditions of frequent use or heavy exposure, respiratory protection may be needed. Respiratory protection is ranked in order from

SECTION 11 TOXICOLOGICAL INFORMATION

FORMAMIDE:
IRRITATION DATA:
100 mg eyes-rabbit severe
TOXICITY DATA:
>3900 ppm/6 hour(s) inhalation-rat LC50; 17 gm/kg skin-rabbit LD50; 5577 mg/kg oral-rat LD50
LOCAL EFFECTS:
Irritant: inhalation, skin, eye
ACUTE TOXICITY LEVEL:
Slightly Toxic: dermal absorption, ingestion

Additional toxicological data is available on the component(s) of this product. Please call 650 554-2860 or contact hazcom@appliedbiosystems.com for more information.

SECTION 12 ECOLOGICAL INFORMATION

Not available

SECTION 13 DISPOSAL CONSIDERATIONS

Dispose in accordance with all applicable regulations.

SECTION 14 TRANSPORT INFORMATION

U.S. DEPARTMENT OF TRANSPORTATION: No classification assigned.

CANADIAN TRANSPORTATION OF DANGEROUS GOODS: No classification assigned.

LAND TRANSPORT ADR: No classification assigned.

LAND TRANSPORT RID: No classification assigned.

AIR TRANSPORT IATA: No classification assigned.

AIR TRANSPORT ICAO: No classification assigned.

MARITIME TRANSPORT IMDG: No classification assigned.

TAB 28

Printed by: Tao Li

From: "Susswein, Lisa" <Lisa_Susswein@med.unc.edu>
Date: Thu, 8 Jul 2004 16:10:16 -0400
Subject: RE: VKOR study
To: "'Darrel Stafford'" <dws@email.unc.edu>, taoli@email.unc.edu
Cc: "'Lisa_Gatti@med.unc.edu'" <Lisa_Gatti@med.unc.edu>

Hi Darrel and Tao,

This is no problem. I just need to get the medical record numbers from Lisa Gatti, and I will forward along the data. If I remember correctly, do you prefer an excel spreadsheet to Access?

Lisa

Lisa Susswein, MS, CGC

Genetic Counselor

Cancer Genetics Network

UNC Chapel Hill

919-843-3158 phone

919-843-7240 fax

susswein@med.unc.edu <mailto:susswein@med.unc.edu>

-----Original Message-----
From: Darrel Stafford [mailto:dws@email.unc.edu]
Sent: Thursday, July 08, 2004 1:43 PM
To: 'Susswein, Lisa'
Subject: RE: VKOR study

Lisa: This is Darrel. I saw that Tao had written you (actually at my behest) to get the doses on the latest patients that he has. He already has them genotyped so that I would like to add them to a slide that I am going to present next week at the Gordon conference-soo if you have the doses of these patients and it is not too much hassle I would like to get them before I leave-Thanks

Darrel Stafford

-----Original Message-----
From: Susswein, Lisa [mailto:Lisa_Susswein@med.unc.edu]
Sent: Monday, June 14, 2004 3:18 PM
To: Evans, Jim; Darrel Stafford; taoli@email.unc.edu; 1lisa Susswein (susswein@med.unc.edu); Skrzynia, Cecile; Lisa Gatti (lgatti@med.unc.edu)
Subject: RE: VKOR study

The progress today: 8 patients who have not yet been approached have agreed to let us pitch the study to them. So we should have some samples for you soon.

Lisa

Printed by: Tao Li

-----Original Message-----
From: Evans, Jim [mailto:jpevans@med.unc.edu]
Sent: Monday, June 14, 2004 3:10 PM
To: Darrel Stafford; taoli@email.unc.edu; 1lisa Susswein (susswein@med.unc.edu); Skrzynia, Cecile
Subject: VKOR study

Hey Darrel and Tao,

Lisa let me know that we just got IRB approval to start calling the folks whom we have been unable to catch in coag clinic. That was the hold-up, but now that we have approval more samples should be coming soon.

I also have calls in to the cardiology clinic director to see if we can start assaulting (er...I mean asking) their patients for permission to be in the study.

Jim

James P. Evans MD, Ph.D

Director of Clinical Cancer Genetics and The Program in Human Genetics

Departments of Genetics and Medicine

CB#7264

University of North Carolina at Chapel Hill

Chapel Hill, NC

27599-7264

Phone: 919 843-6475

Fax: 919 843-4682

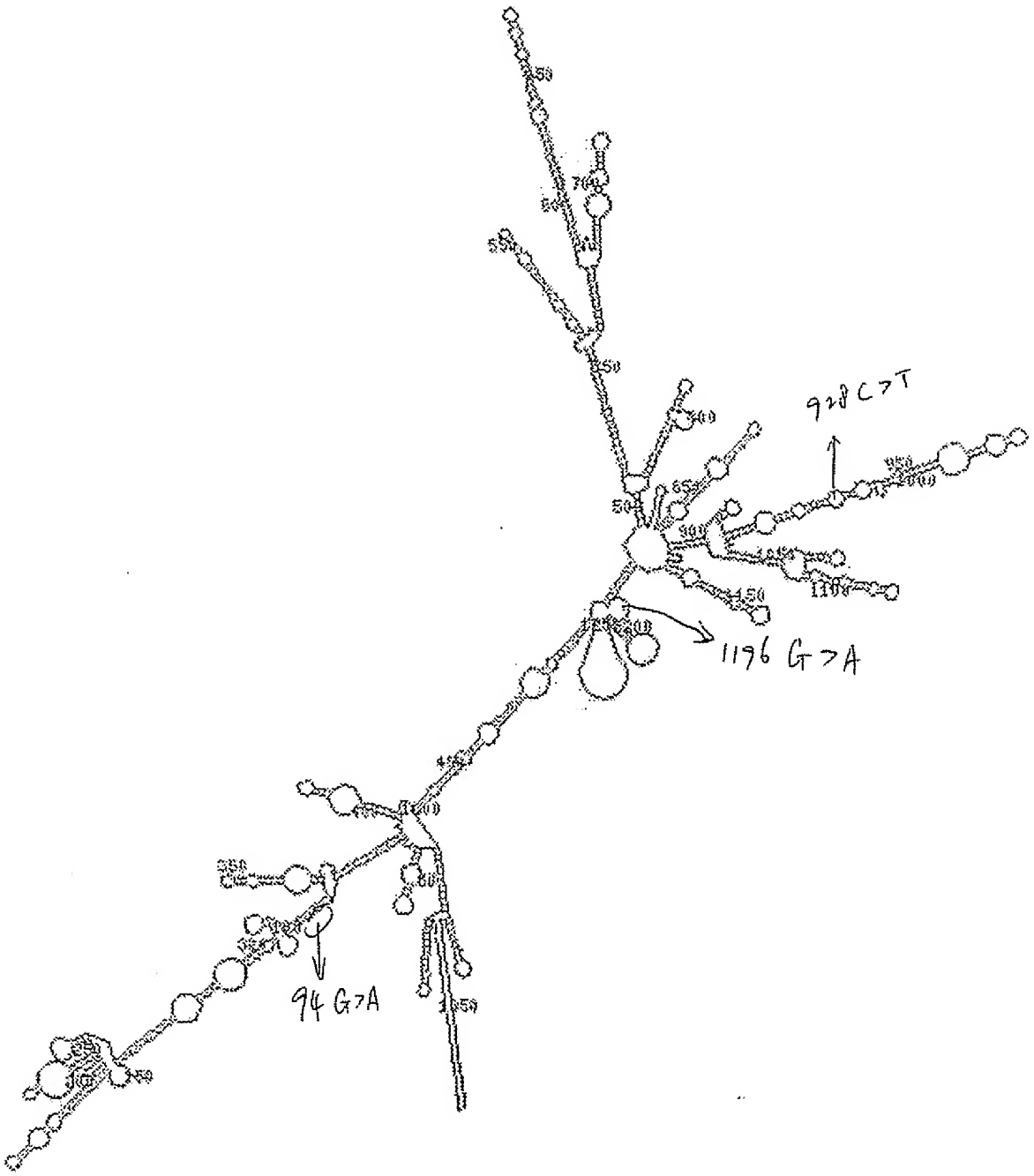
email: jpevans@med.unc.edu

There is 1 attachment in this message:

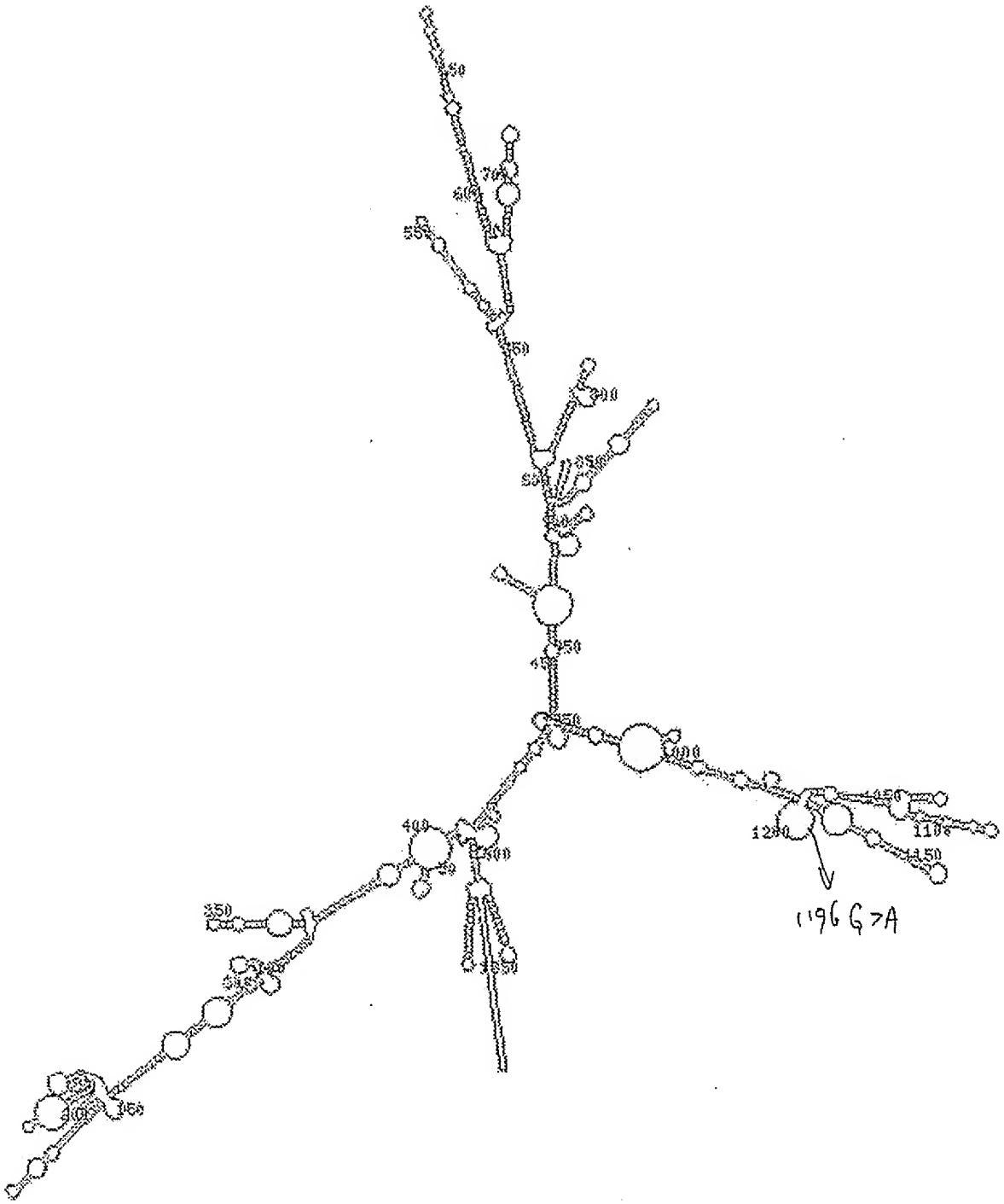
Attachment 1 - 12719 bytes
Content Type : TEXT/HTML

TAB 29

plt22jpg by D. Stewart and M. Zuker

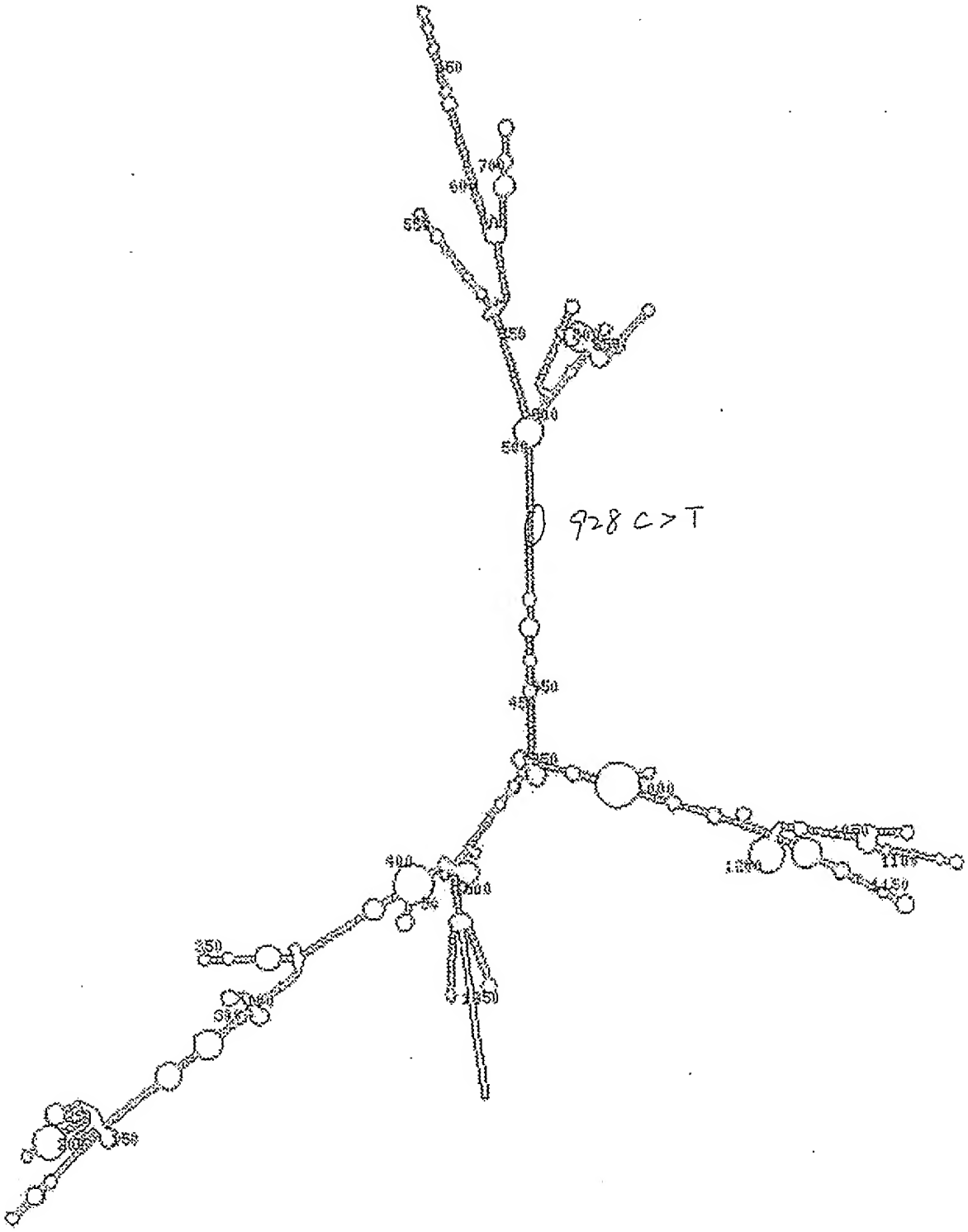


dG = -529.97 [initially -544.0] wild type



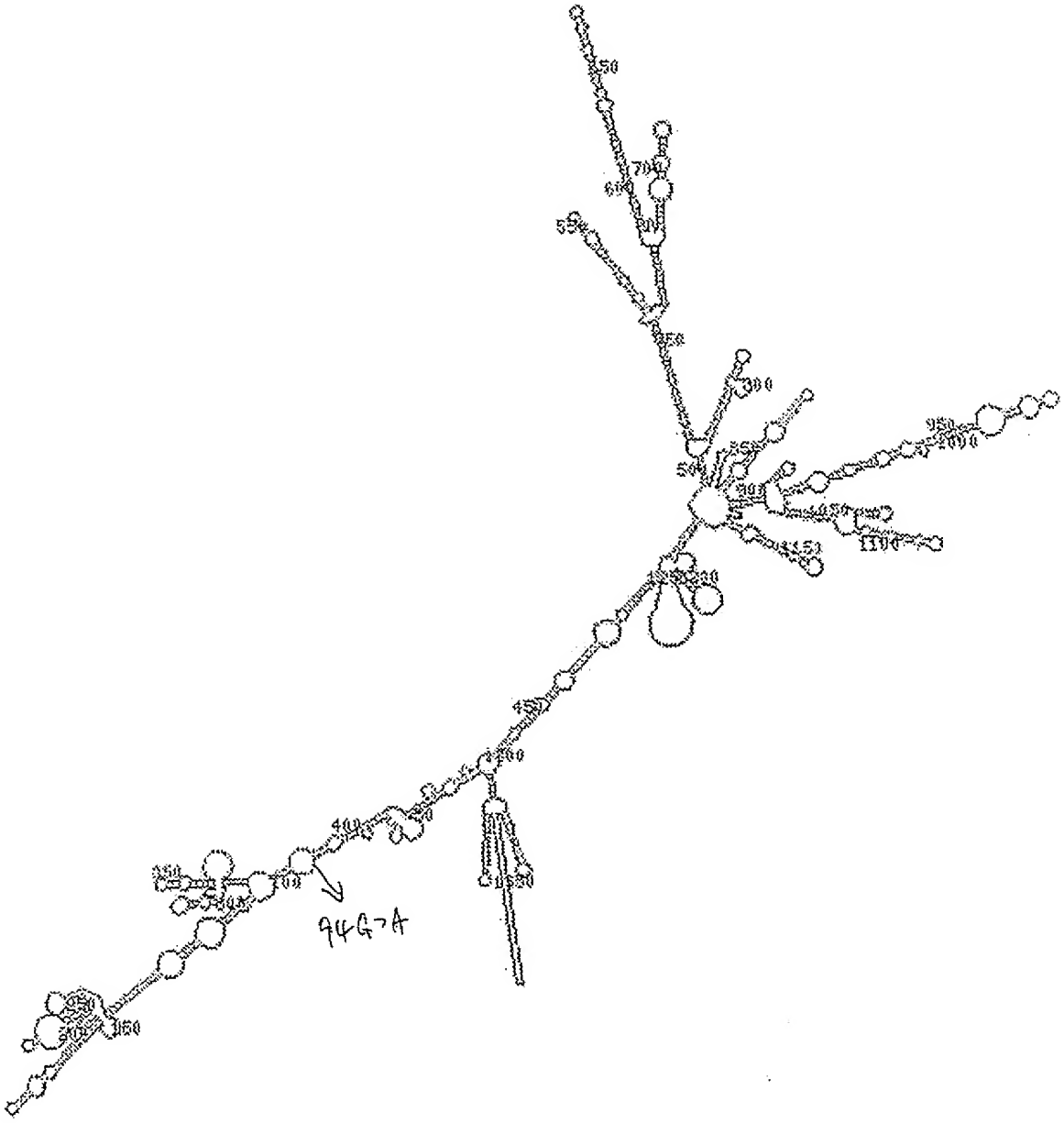
dG = -509.92 [initially -541.5] 3' UTR

plt22jpg by D. Stewart and M. Zuker



dG = -516.7 [initially -544.1] exon 3

plt22jpg by D. Stewart and M. Zuker



dG = -519.81 [initially -542.01] 5'UTR

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Mon, 12 Jul 2004 13:46:40 -0400 (Eastern Daylight Time)
Subject: From Tao: VKOR SNPs mRNA structure
To: dws@email.unc.edu

-- Begin original message --

> From: "Shabalina, Svetlana (NIH/NLM/NCBI)" <shabalin@ncbi.nlm.nih.gov>
> Date: Mon, 12 Jul 2004 13:05:40 -0400
> Subject: RE: From Tao: VKOR SNPs
> To: "'taoli@email.unc.edu'" <taoli@email.unc.edu>
>
> Dear Tao,
> I am sending to you the most stable structures for all four sequences which
> I have from you. They are different from wild type structure in positions 94
> (G/A in 5'UTR), 928 (C/T in 3 exon) and 1196 (G/A in 3' UTR)
> correspondingly. I have not analyzed suboptimal structures yet, it takes
> more time. Probably, the SNP (C/T) in position 928 influences dramatically
> local stem-loop structures. ACCC- motif in 3'UTR might influence the mRNA
> stability or probably binds a protein (see Motif1.txt).
> Regards,
> Svetlana
>
>
> -----Original Message-----
> From: taoli@email.unc.edu [mailto:taoli@email.unc.edu]
> Sent: Tuesday, July 06, 2004 12:38 PM
> To: Shabalina, Svetlana (NIH/NLM/NCBI)
> Subject: RE: From Tao: VKOR SNPs
>
> Hi! Dear Sveta,
>
> How was your vacation? I am sorry to bother you immediately after you are
> back.
> My boss will go to a conference on July 8 and he hopes to put a slide about
> the RNA stability in his presentation before he leave. If you have some
> free time these days, could you please help us analyze the VKOR mutations
> first (the Septin is not very important)?
>
> Thank you very much!
>
> Tao
> 919-360-8663
>
>
> -- Begin original message --
>
> > From: "Shabalina, Svetlana (NIH/NLM/NCBI)" <shabalin@ncbi.nlm.nih.gov>
> > Date: Sat, 26 Jun 2004 21:14:36 -0400
> > Subject: RE: From Tao: VKOR SNPs
> > To: "'taoli@email.unc.edu'" <taoli@email.unc.edu>
> >
> > Hi Tao,
> > I 'll be glad to predict the RNA secondary structure and analyse
> > degradation motifs, when I'll be back to Bethesda.
> > I am out of town till July 5th. Sorry for the delay with the answer.
> > Regards,
> > Sveta
> >
> > -----Original Message-----
> > From: taoli@email.unc.edu [mailto:taoli@email.unc.edu]
> > Sent: Tuesday, June 15, 2004 6:07 PM
> > To: Shabalina, Svetlana (NIH/NLM/NCBI)
> > Subject: From Tao: VKOR SNPs
> >
> > Hi! Sveta,
> >
> > Last Friday I talked with Luda about my data. We found that a 3'-UTR
> > SNP of VKOR gene has strong relationship with the warfarin dosage.
> > She suggest I try to get help from you, who is an excellent molecular
> > specialist, to predict the RNA secondary structure and degradation motifs.
> > Besides the 3'-UTR SNP, we also found that other 2 SNPs which are in

> > Exon 3 and 5'-UTR are also interesting.
> >
> > Besides, a person in my group is doing SNPs of Septin5. If it would
> > not cause you much trouble, could you please also analyse them for me?
> >
> >
> > Thank you ahead of time for your help!
> >
> > Tao
> > tel: 919-360-8663 (cell)
> >
> >
> >
>
> -- End original message --
>
>

-- End original message --

There are 5 attachments in this message:

Attachment 1 - 40150 bytes
Content Type : IMAGE/JPEG

Attachment 2 - 40430 bytes
Content Type : IMAGE/JPEG

Attachment 3 - 38110 bytes
Content Type : IMAGE/JPEG

Attachment 4 - 41256 bytes
Content Type : IMAGE/JPEG

Attachment 5 - 3922 bytes
Content Type : TEXT/PLAIN

TAB 30

Printed by: Tao Li

From: taoli@email.unc.edu (Tao Li)
Date: Wed, 14 Jul 2004 14:56:44 -0400 (Eastern Daylight Time)
Subject: VKOR SNPs: new results
To: jpevans@med.unc.edu ("Evans, Jim")
Cc: dws@email.unc.edu

Hi! Jim,

There are some good results for VKOR SNPs recently.

I sent the VKOR mRNA sequence with 3'UTR, 5'UTR and Leu120Leu SNPs to NIH to predict the mRNA secondary structure and the results came back this week. The 5'UTR change doesn't affect much on the mRNA structure, however, the 3'UTR G to A switch changes the mRNA secondary structure a lot and it might affect the mRNA stability, besides, a ACCC motif adjacent to the G>A might bind to a RNA binding protein and the nucleotide change may cause the mRNA stability change. Leu120Leu SNP, which was found in patient #3 (the very special one), changes the mRNA local structure dramatically. There is a recent paper in Nature Genetics reported that a heterozygous change in TGFBR2 (synonymous AA substitution of Q508Q) causes Marfan syndrome. The Leu120Leu in VKOR might also be important for warfarin dosage.

Now we have total 58 patients. I have tested the genotype on the 3'UTR SNP for patient #48-58, and the trends of warfarin dosage still follows the pattern of widetype<heterozygous<homozygous. I also genotyped other 2 SNPs in intron2, and the results are very interesting: both of them are also apparently with the same pattern of widetype<heterozygous<homozygous. Besides, the 2 SNPs seem to link together often. I will have my wife to do more staticstic work to examine the inflence of other factors and their combinations.

These results sound exciting to me. However, I think more patient will make the results more valid. Hopefully we can find other ways to recruit more patients in a short time. The current rate of ~10 patients/month is a little slower than I expected, though I understand that Lisa and Besty already tried their best.

Darrel is out of town these days. May we have a meeting together when he comes back? Next Thursday 10am I will give a talk in my labmeeting. If you have time, could you please come over to join us and we can talk about the details after our labmeeting? If not, then we may pick another time.

Have a nice day!

Tao

TAB 31

SNP_3UTR SNP_int2_1

Frequency / Row Pct	Wildtype	Hetero	Homo	Total
Wildtype	4 14.81	8 29.63	15 55.56	27
Hetero	10 55.56	8 44.44	0 0.00	18
Homo	11 91.67	1 8.33	0 0.00	12
Total	25	17	15	57

Fisher's Exact Test

Table Probability (P)	8.044E-10
Pr <= P	3.302E-07

Sample Size = 57

Table of SNP_int2_1 by SNP_int2_2

SNP_int2_1 SNP_int2_2

Frequency / Row Pct	Wildtype	Hetero	Homo	Total
Wildtype	0 0.00	3 12.00	22 88.00	25
Hetero	0 0.00	17 100.00	0 0.00	17
Homo	11 73.33	4 26.67	0 0.00	15
Total	11	24	22	57

Fisher's Exact Test

Table Probability (P)	2.156E-18
Pr <= P	6.241E-18

Table of SNP_3UTR by SNP_int2_2

SNP_3UTR SNP_int2_2

Frequency / Row Pct	Wildtype	Hetero	Homo	Total
Wildtype	11 40.74	12 44.44	4 14.81	27
Hetero	0 0.00	11 61.11	7 38.89	18
Homo	0 0.00	1 8.33	11 91.67	12
Total	11	24	22	57

Fisher's Exact Test

Table Probability (P)	6.224E-09
Pr <= P	2.758E-06

Average of Variables:

---------- SNP_3UTR=' Wildtype' ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error

AVG_Dose	AVG_Dose	35.3520622	20.8269315	4.0081448
logdose		3.4247604	0.5394948	0.1038258
AVG_INR	AVG_INR	2.5566674	0.4786871	0.0921234

---------- SNP_3UTR=Hetero ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	41.3028005	16.1586302	3.8086257
logdose		3.6335933	0.4520804	0.1065564
AVG_INR	AVG_INR	2.5813610	0.4145982	0.0977217

---------- SNP_3UTR=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	47.5609630	13.3799866	3.8624694
logdose		3.8273259	0.2721294	0.0785570
AVG_INR	AVG_INR	2.3191117	0.3681276	0.1062693

show the mean of average INR and Dose by snp_intron2_1

---------- SNP_int2_1=' Wildtype' ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	48.1336496	12.7368756	2.5473751
logdose		3.8383733	0.2776236	0.0555247
AVG_INR	AVG_INR	2.3521196	0.4147968	0.0829594

---------- SNP_int2_1=Hetero ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	35.1921249	15.3860425	3.7316634
logdose		3.4646447	0.4633535	0.1123797
AVG_INR	AVG_INR	2.6694068	0.4469707	0.1084063

---------- SNP_int2_1=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	31.1386855	24.0234425	6.2028262
logdose		3.2628553	0.5734644	0.1480679
AVG_INR	AVG_INR	2.6093968	0.4165374	0.1075495

show the mean of average INR and Dose by SNP_intron2_2:

---------- SNP_int2_2=' Wildtype' ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	25.2927392	10.1090598	3.0479962
logdose		3.1442028	0.4606249	0.1388836
AVG_INR	AVG_INR	2.6769791	0.4114026	0.1240426

---------- SNP_int2_2=Hetero ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	39.1814767	21.6262136	4.4144324
logdose		3.5406090	0.5115253	0.1044147
AVG_INR	AVG_INR	2.6169731	0.4378687	0.0893796

---------- SNP_int2_2=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	47.7323670	12.9025178	2.7508260
logdose		3.8291034	0.2812767	0.0599684
AVG_INR	AVG_INR	2.3213516	0.4077631	0.0869354

If swap the definition of wild type and homo in snp at intron2_3294

```
if SNP_3294=2 then SNP_int2_2_2=' Wildtype';
if SNP_3294=1 then SNP_int2_2_2='Hetero';
if SNP_3294=0 then SNP_int2_2_2='Homo';
```

Table of SNP_int2_1 by SNP_int2_2_2

SNP_int2_1	SNP_int2_2_2			
Frequency Row Pct	Wildtype	Hetero	Homo	Total
Wildtype	22 88.00	3 12.00	0 0.00	25
Hetero	0 0.00	17 100.00	0 0.00	17
Homo	0 0.00	4 26.67	11 73.33	15
Total	22	24	11	57

Fisher's Exact Test

Table Probability (P)	2.156E-18
Pr <= P	6.241E-18

TAB 32

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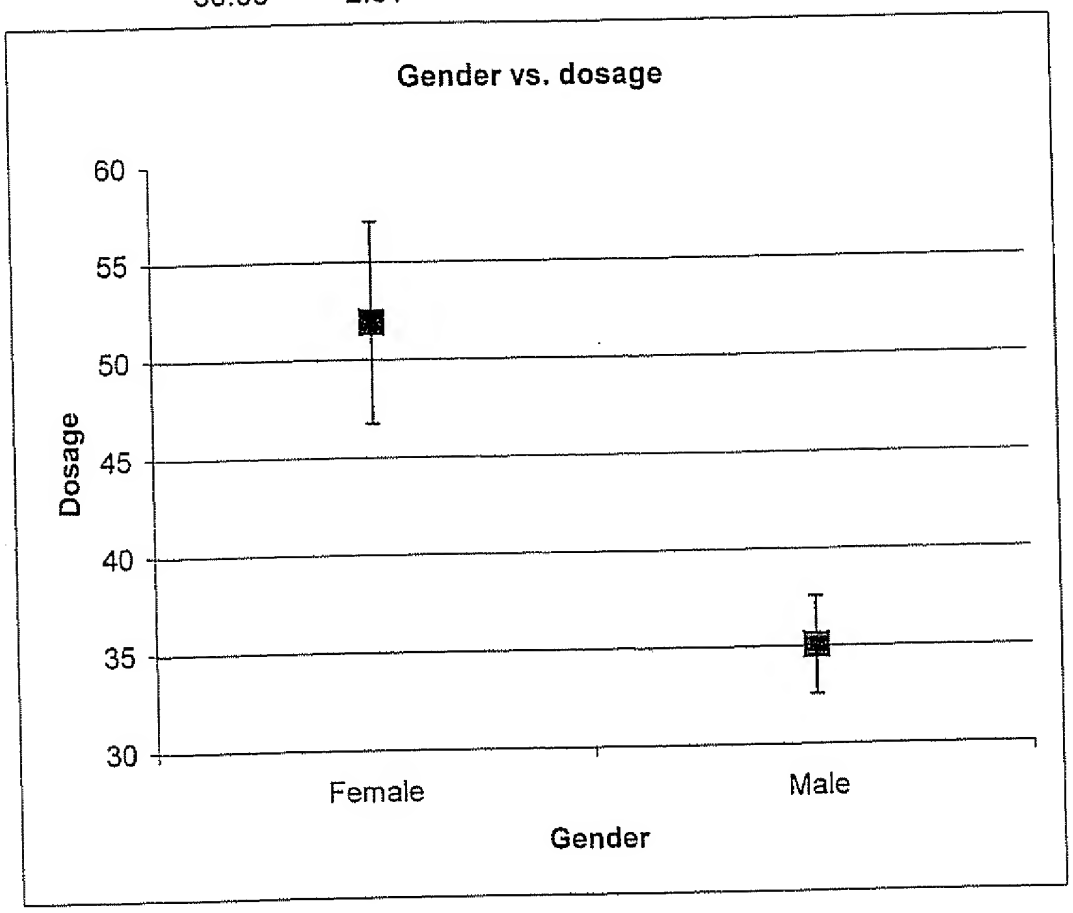
UNCStudyNo	Gender	Race	SNP_4769	SNP_2581	SNP_3294	AVG_INR	AVG_Dose
1	Male	African American	0	0	2	2.46	43.30
2	Male	Caucasian	0	2	0	3.01	16.90
3	Female	African American	1	0	1	2.83	101.67
4	Male	African American	1	1	1	2.88	24.17
5	Male	Caucasian	0	0	2	2.18	68.13
6	Male	Caucasian	0	2	0	2.20	37.36
7	Male	Caucasian	1	1	1	2.59	33.11
8	Male	Caucasian	0	1	1	3.31	49.63
9	Male	Caucasian	2	0	2	2.36	65.96
10	Female	African American	0	2	1	2.59	24.22
11	Male	Caucasian	0	2	0	2.46	16.31
12	Male	Caucasian	2	0	2	2.62	33.50
13	Female	Caucasian	2	0	2	2.58	57.21
14	Male	Caucasian	0	2	1	2.92	18.18
15	Female	Caucasian	1	0	2	2.07	63.31
16	Male	Caucasian	0	2	0	2.36	24.13
17	Male	Caucasian	1	0	2	2.21	48.00
18	Male	African American	0	2	1	2.25	35.00
19	Male	Caucasian	0	2	0	3.18	38.46
20	Female	Caucasian	1	1	1	2.28	22.76
21	Female	Caucasian	1	0	2	2.77	50.00
22	Male	Caucasian	1	0	2	2.27	24.77
23	Male	Caucasian	0	2	0	2.09	24.24
24	Female	African American	0	2	1	1.93	111.46
25	Male	Caucasian	1	0	2	2.15	40.28
26	Male	Caucasian	2	0	2	2.48	41.67
27	Female	African American	1	0	1	2.14	35.42
28	Female	Caucasian	1	1	1	2.74	56.77
29	Female	Caucasian	0	2	0	2.94	31.11
30	Male	Caucasian	0	1	1	2.50	13.81

31	Male	African American	2	0	2	2.51	46.45
32	Male	Caucasian	2	1	1	2.30	31.94
33	Female	Caucasian	0	1	1	3.17	62.91
34	Male	Caucasian	1	1	1	2.74	29.00
35	Female	Caucasian	0	1	1	2.43	33.97
36	Male	African American	0	2	0	2.34	8.62
37	Male	African American	1	0	1	3.09	58.65
38	Male	African American	2	0	2	2.90	40.33
39	Female	Caucasian	2	0	2	2.19	64.67
40	Male	Caucasian	0	1	1	1.80	35.77
41	Male	Caucasian	1	1	1	2.71	16.53
42	Male	Caucasian	0	2	0	2.72	25.02
43	Male	Caucasian	0	1	1	3.20	24.06
44	Female	Caucasian	0	2	0	3.31	38.58
45	Female	Caucasian	0	1	1	2.08	46.25
46	Female	Caucasian	0	0	2	2.72	27.86
47	Male	Caucasian	0	1	1	2.56	41.75
48	Female	Caucasian	1	0	2	3.18	51.76
49	Female	Caucasian	1	0	1	2.50	59.17
50	Female	Caucasian	1	1	1	2.61	59.19
51	Male	Caucasian	2	0	2	1.83	70.00
52	Male	African American	2	0	2	1.63	43.00
53	Female	Caucasian	0	0	2	1.50	40.00
54	Male	Caucasian	1	1	1	3.50	16.66
55	Male	Caucasian	1	0	2	2.04	53.93
56	Male	Caucasian	0	2	0	2.84	17.50
57	Male	Caucasian	2	0	2	2.52	40.00
58	Male	Caucasian	2	0	2	1.92	36.00

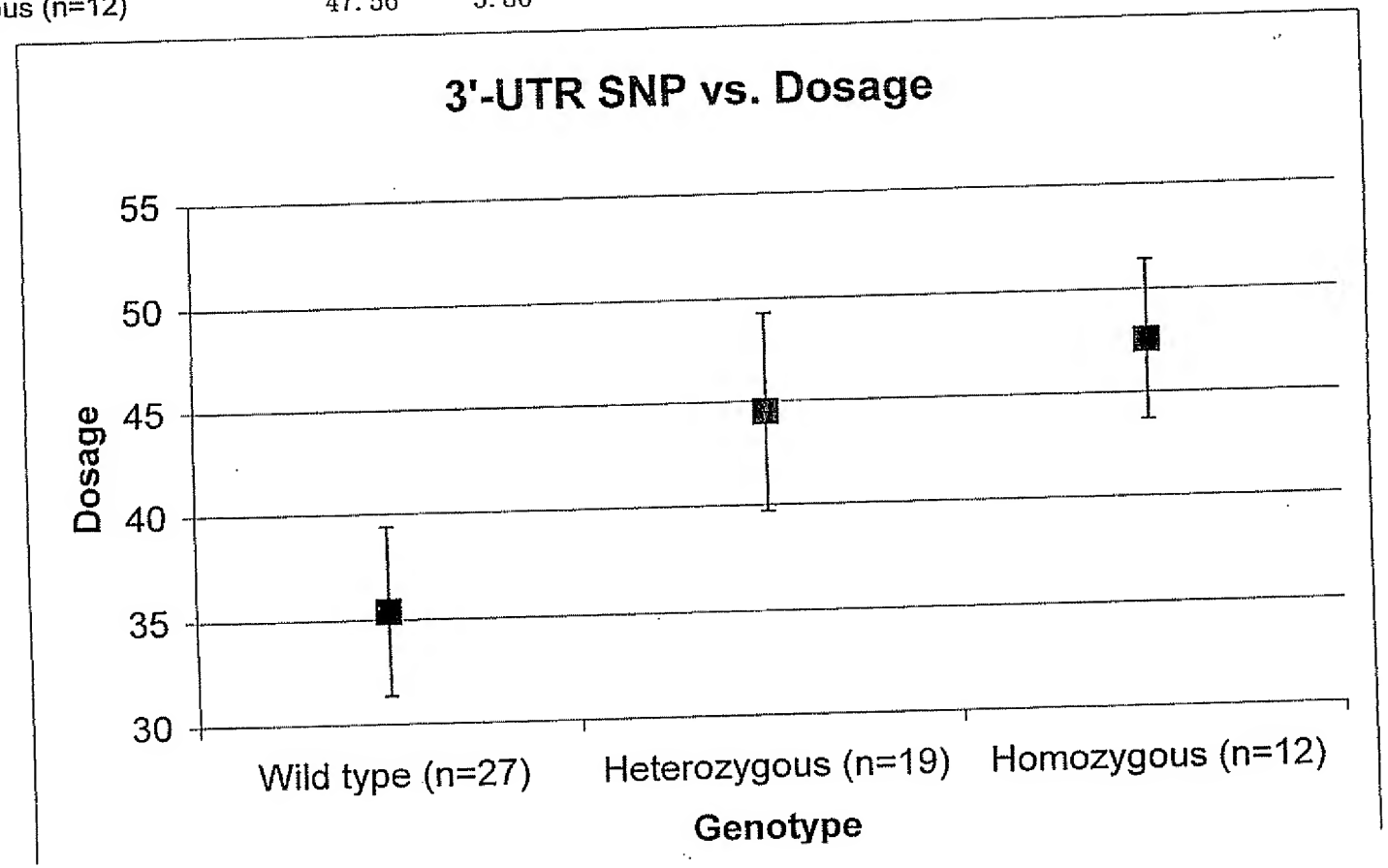
Gender vs. Dosage

Gender	Average Dose	Std Error
Female	51.91	5.17
Male	35.06	2.51



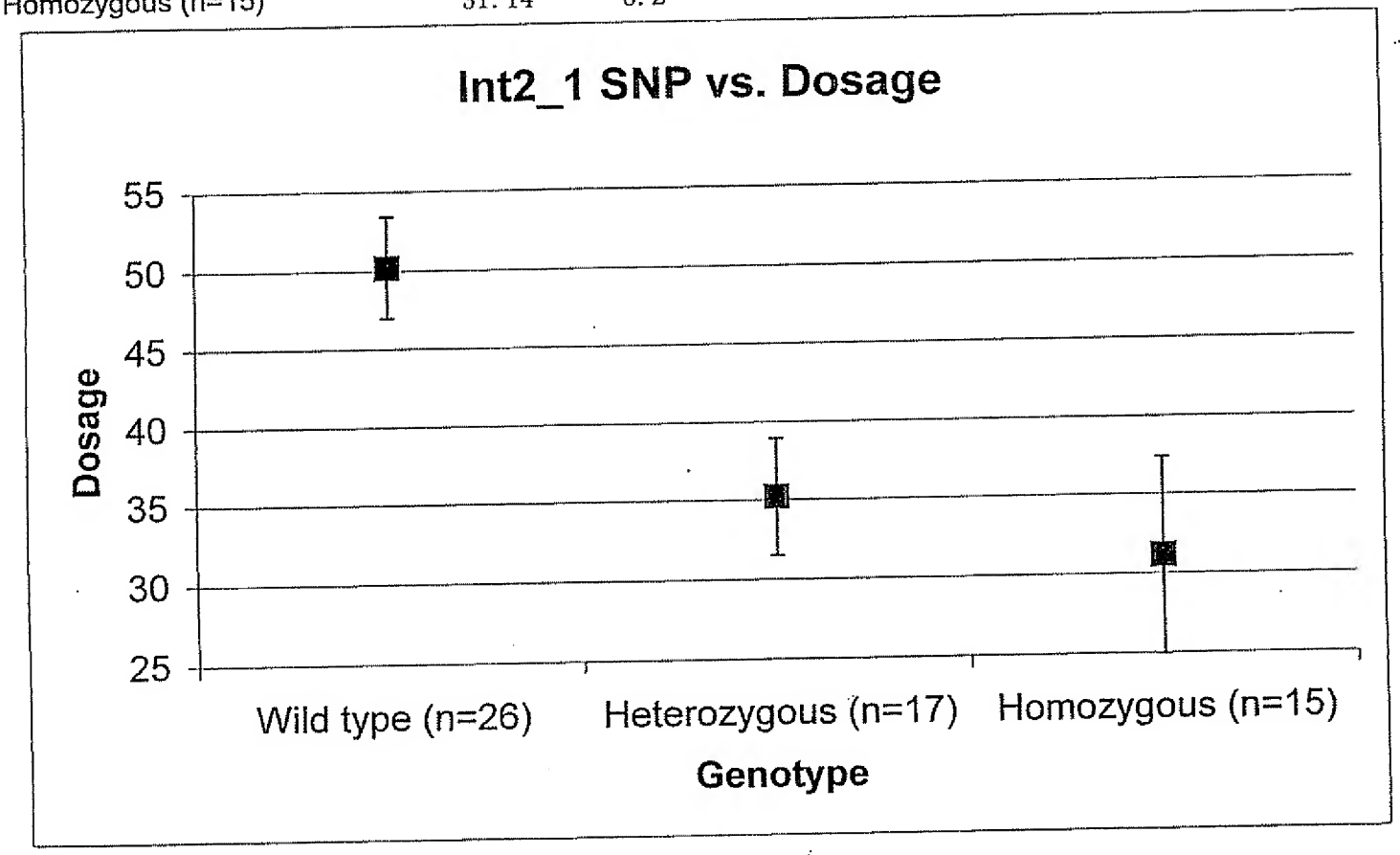
3'-UTR SNP vs. Dosage

Genotype	Average Dose	Std Error
Wild type (n=27)	35.35	4.01
Heterozygous (n=19)	44.48	4.8
Homozygous (n=12)	47.56	3.86



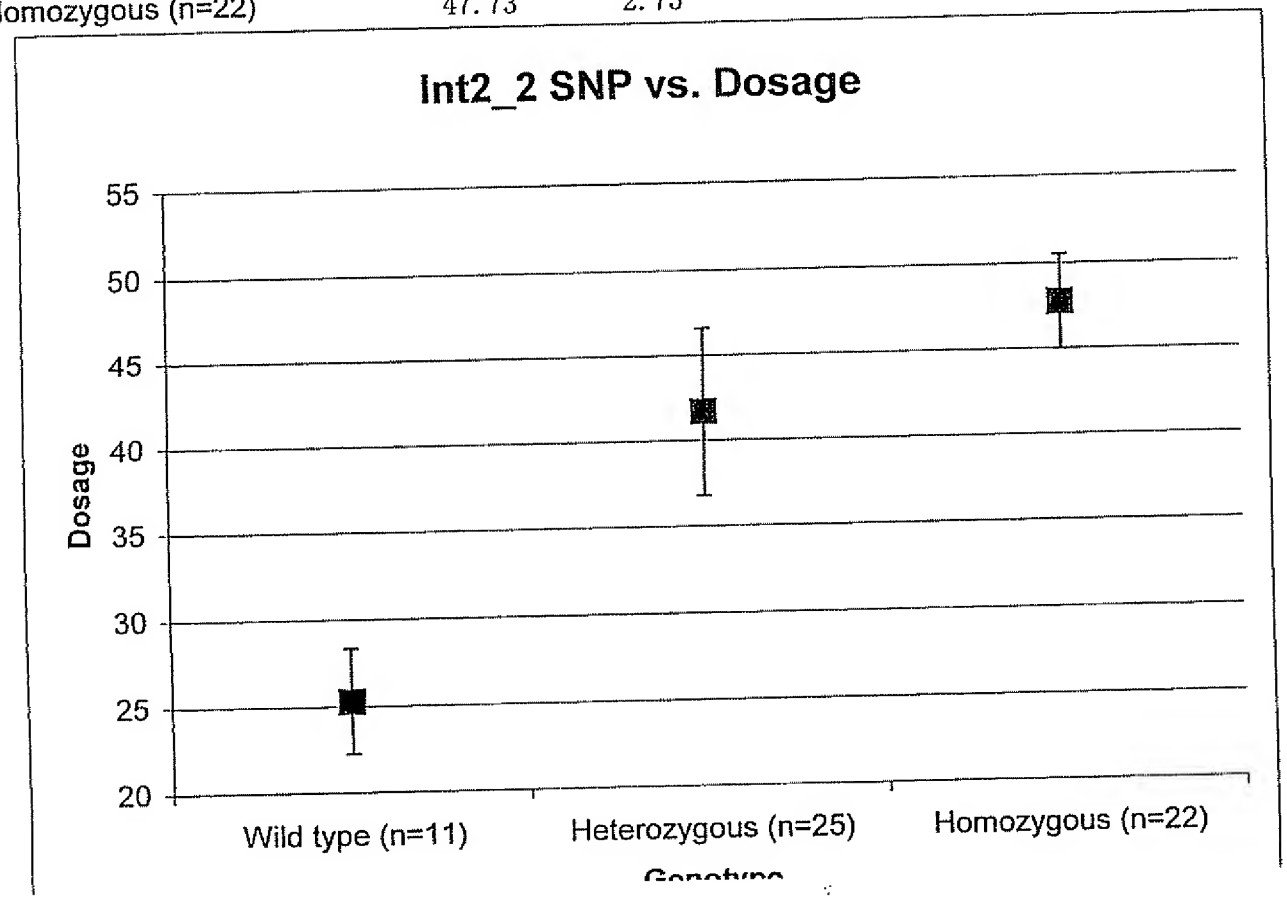
Int2_1 SNP vs. Dosage

Genotype	Average Dose	Std Error
Wild type (n=26)	50.19	3.2
Heterozygous (n=17)	35.19	3.73
Homozygous (n=15)	31.14	6.2



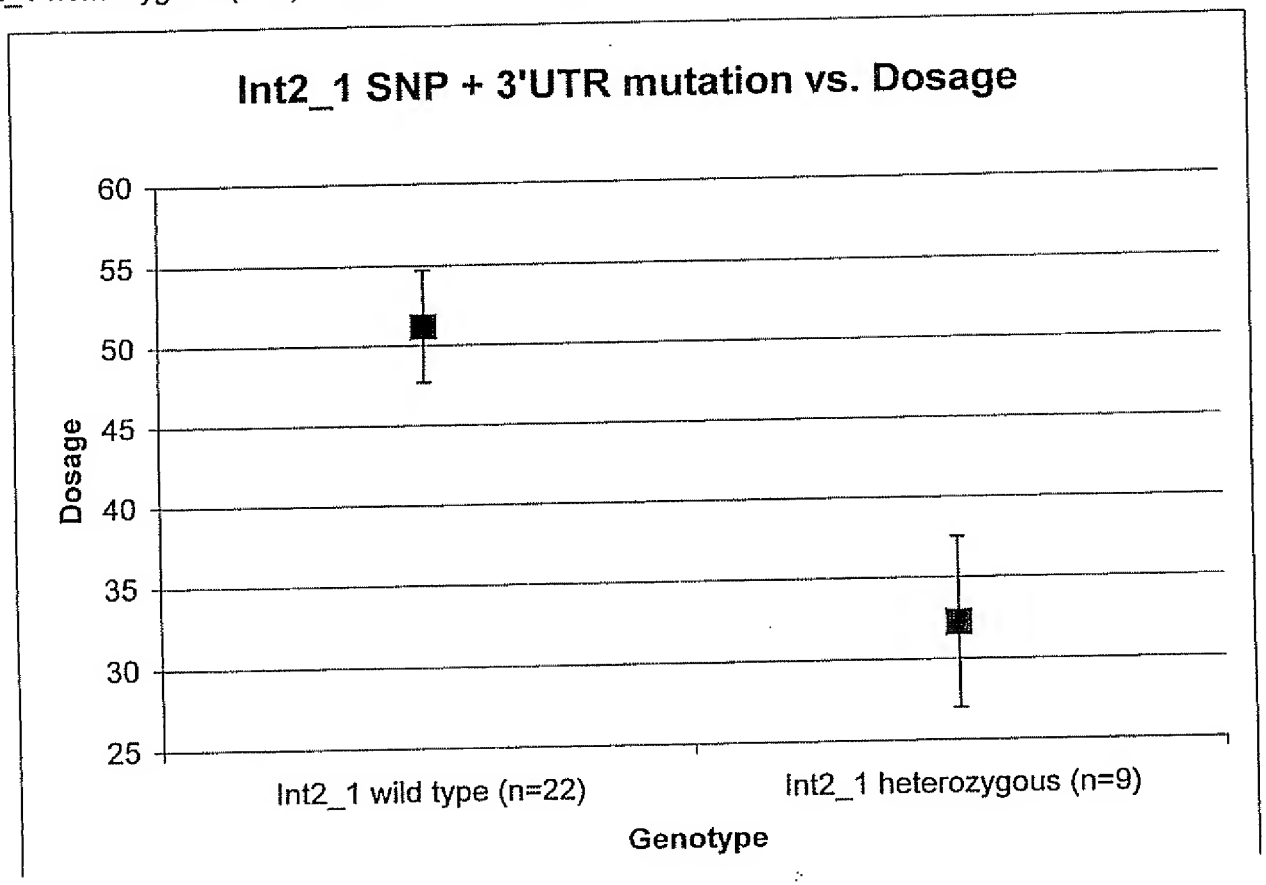
Int2_2 SNP vs. Dosage

Genotype	Average Dose	Std Error
Wild type (n=11)	25.29	3.05
Heterozygous (n=25)	41.68	4.92
Homozygous (n=22)	47.73	2.75



Int2_1 SNP + 3'UTR mutation vs. Dosage

Genotype	Average Dose	Std Error
Int2_1 wild type (n=22)	51.17	3.5
Int2_1 heterozygous (n=9)	32.24	5.25
Int2_1 homozygous (n=0)	N/A	N/A



SNP_Int2_1 vs. SNP_Int2_2

		SNP_Int2_2			
		wildtype	hetero	homo	Total
SNP_Int2_1	wildtype	0	4	22	26
	hetero	0	17	0	17
	homo	11	4	0	15
	Total	11	25	22	58

SNP_Int2_1 vs. SNP_3'UTR

		SNP_3'UTR			
		wildtype	hetero	homo	Total
SNP_Int2_1	wildtype	4	11	11	26
	hetero	8	8	1	17
	homo	15	0	0	15
	Total	27	19	12	58

SNP_Int2_2 vs. SNP_3'UTR

		SNP_3'UTR			
		wildtype	hetero	homo	Total
SNP_Int2_2	wildtype	11	0	0	11
	hetero	12	12	1	25
	homo	4	7	11	22
	Total	27	19	12	58

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```
if (SNP_2581=2 and SNP_3294=0 and SNP_4769=0) then SNP_type=1;
if (SNP_2581=0 and SNP_3294=2 and SNP_4769=2) then SNP_type=2;
if (SNP_2581=0 and SNP_3294=2 and SNP_4769=0) then SNP_type=3;
```

The GLM Procedure

Level of SNP_int2_1	Level of SNP_int2_2	Level of SNP_3UTR	N	AVG_Dose Mean	AVG_Dose Std Dev	logdose Mean	logdose Std Dev
Wildtype	Hetero	Hetero	4	63.7247917	27.6149784	4.08519741	0.43066602
Wildtype	Homo	Wildtype	4	44.8214286	16.8956947	3.75138706	0.36745944
Wildtype	Homo	Hetero	7	47.4341781	12.1429376	3.82440204	0.30296339
Wildtype	Homo	Homo	11	48.9806465	13.0507406	3.86035564	0.25895767
Hetero	Hetero	Wildtype	8	38.5174603	15.2901075	3.56501912	0.47523865
Hetero	Hetero	Hetero	8	32.2727495	16.8352557	3.36435102	0.49180566
Hetero	Hetero	Homo	1	31.9444444	.	3.46399828	.
Homo	Wildtype	Wildtype	11	25.2927392	10.1090598	3.14420276	0.46062491
Homo	Hetero	Wildtype	4	47.2150379	43.3901209	3.58914993	0.79615152

ANOVA and Bonferroni (Dunn) t Tests for avg_dose
Comparisons significant at the 0.05 level are indicated by ***

Difference

SNP_type Comparison	Between Means	Simultaneous 95% Confidence Limits		
2 - 3	4.159	-14.653	22.972	
2 - 1	23.688	9.949	37.427	***
3 - 2	-4.159	-22.972	14.653	
3 - 1	19.529	0.716	38.341	***
1 - 2	-23.688	-37.427	-9.949	***
1 - 3	-19.529	-38.341	-0.716	***

ANOVA and Bonferroni (Dunn) t Tests for logdose

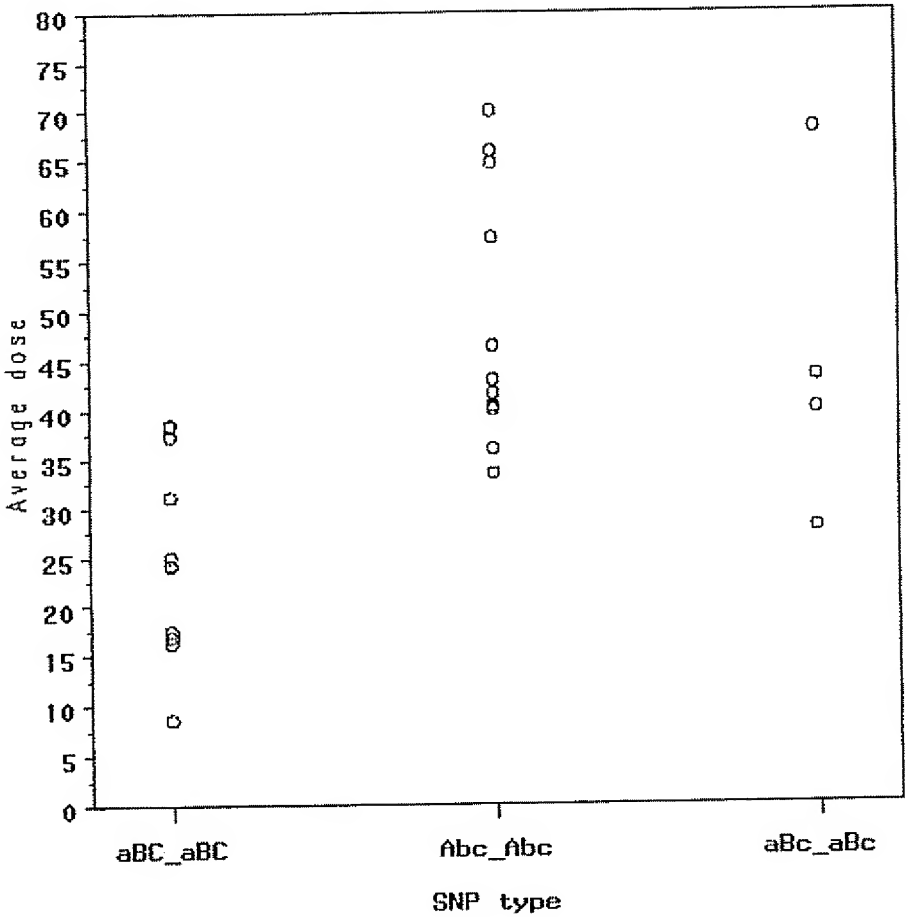
NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than Tukey's for all pairwise comparisons.

Comparisons significant at the 0.05 level are indicated by ***.

SNP_type Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
2 - 3	0.1090	-0.4531	0.6711	
2 - 1	0.7162	0.3057	1.1267	***
3 - 2	-0.1090	-0.6711	0.4531	
3 - 1	0.6072	0.0451	1.1693	***
1 - 2	-0.7162	-1.1267	-0.3057	***
1 - 3	-0.6072	-1.1693	-0.0451	***

```
if (SNP_2581=2 and SNP_3294=0 and SNP_4769=0) then SNP_type=' aBC_aBC';
if (SNP_2581=0 and SNP_3294=2 and SNP_4769=2) then SNP_type='Abc_Abc';
if (SNP_2581=0 and SNP_3294=2 and SNP_4769=0) then SNP_type='aBc_aBc';
```

show the AVG_dose by SNP type



TAB 33

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Gender=Female ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	51.9134706	23.1353057	5.1732116
logdose		3.8622490	0.4271634	0.0955167
AVG_INR	AVG_INR	2.5273414	0.4605295	0.1029775

---------- Gender=Male ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	35.0549535	15.4669393	2.5090689
logdose		3.4520493	0.4847888	0.0786431
AVG_INR	AVG_INR	2.5159745	0.4356922	0.0706786

---------- Race=African American ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	47.6897631	30.3612448	8.7645364
logdose		3.6766722	0.6738239	0.1945162
AVG_INR	AVG_INR	2.4629970	0.4329073	0.1249696

---------- Race=Caucasian ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	39.0887062	16.2625510	2.3977823
logdose		3.5717997	0.4538327	0.0669140
AVG_INR	AVG_INR	2.5347368	0.4458326	0.0657344

Table of Gender by Race

Gender(Gender) Race(Race)

Frequency,	African American,	Caucasian	Total
Female	4	16	20
Male	8	30	38
Total	12	46	58

---------- Gender=Female Race=African American ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	68.1912500	44.7232652	22.3616326
logdose		4.0224361	0.7619808	0.3809904
AVG_INR	AVG_INR	2.3720383	0.4100553	0.2050276

---------- Gender=Female Race=Caucasian ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	47.8440258	13.7701686	3.4425422
logdose		3.8222022	0.3262677	0.0815669
AVG_INR	AVG_INR	2.5661672	0.4764193	0.1191048

---------- Gender=Male Race=African American ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	37.4390197	15.2009395	5.3743437
logdose		3.5037902	0.6018145	0.2127736
AVG_INR	AVG_INR	2.5084764	0.4640534	0.1640677

---------- Gender=Male Race=Caucasian ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	34.4192025	15.7308114	2.8720401
logdose		3.4382518	0.4598858	0.0839633
AVG_INR	AVG_INR	2.5179740	0.4361127	0.0796229

---------- SNP_3UTR=' Wildtype' ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	35.3520622	20.8269315	4.0081448
logdose		3.4247604	0.5394948	0.1038258
AVG_INR	AVG_INR	2.5566674	0.4786871	0.0921234

---------- SNP_3UTR=Hetero ----------

Variable	Label	Mean	Std Dev	Std Error

AVG_Dose	AVG_Dose	44.4800216	20.9378995	4.8034836
logdose		3.6856006	0.4943813	0.1134189
AVG_INR	AVG_INR	2.5944473	0.4069347	0.0933572

---------- SNP_3UTR=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	47.5609630	13.3799866	3.8624694
logdose		3.8273259	0.2721294	0.0785570
AVG_INR	AVG_INR	2.3191117	0.3681276	0.1062693

SNP_3UTR	Frequency	Percent	Frequency	Percent
Wildtype	27	46.55	27	46.55
Hetero	19	32.76	46	79.31
Homo	12	20.69	58	100.00

---------- Gender=Female SNP_3UTR=' Wildtype' ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	46.2610560	26.9978119	8.9992706
logdose		3.7231340	0.4662912	0.1554304
AVG_INR	AVG_INR	2.5182820	0.5979937	0.1993312

---------- Gender=Female SNP_3UTR=Hetero ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	55.5601893	21.6234871	7.2078290
logdose		3.9467524	0.4124242	0.1374747
AVG_INR	AVG_INR	2.5687445	0.3595494	0.1198498

---------- Gender=Female SNP_3UTR=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	60.9391026	5.2715717	3.7275641
logdose		4.1080007	0.0866137	0.0612451
AVG_INR	AVG_INR	2.3817949	0.2759530	0.1951282

---------- Gender=Male SNP_3UTR=' Wildtype' ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	29.8975653	15.0296770	3.5425288

---------- SNP_int2_2=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	47.7323670	12.9025178	2.7508260
logdose		3.8291034	0.2812767	0.0599684
AVG_INR	AVG_INR	2.3213516	0.4077631	0.0869354

The FREQ Procedure

SNP_int2_2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Wildtype	11	18.97	11	18.97
Hetero	25	43.10	36	62.07
Homo	22	37.93	58	100.0

show the mean of average INR and Dose by SNP_int2_2

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---------- Gender=Female SNP_int2_2=' Wildtype' ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	34.8440171	5.2791263	3.7329060
logdose		3.5451096	0.1520910	0.1075446
AVG_INR	AVG_INR	3.1250000	0.2553441	0.1805556

---------- Gender=Female SNP_int2_2=Hetero ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	55.7983320	28.8930755	8.7115900
logdose		3.9014736	0.5186233	0.1563708
AVG_INR	AVG_INR	2.4811787	0.3632504	0.1095241

---------- Gender=Female SNP_int2_2=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	50.6856753	13.1297323	4.9625724
logdose		3.8912214	0.2972416	0.1123468
AVG_INR	AVG_INR	2.4291232	0.5537135	0.2092840

---------- Gender=Male SNP_int2_2=' Wildtype' ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	23.1702330	9.8174756	3.2724919
logdose		3.0551123	0.4617541	0.1539180
AVG_INR	AVG_INR	2.5774189	0.3769392	0.1256464

---------- Gender=Male SNP_int2_2=Hetero ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	30.5888421	13.1344327	3.5103248
logdose		3.3342956	0.4357924	0.1164704
AVG_INR	AVG_INR	2.7388849	0.4578145	0.1223561

---------- Gender=Male SNP_int2_2=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	46.3541564	13.0167169	3.3609019
logdose		3.8001150	0.2792487	0.0721017
AVG_INR	AVG_INR	2.2710581	0.3308916	0.0854358

show the catagory of gender and snp_intron2_2

show the catagory of gender and snp_intron2_2

Table of Gender by SNP_int2_2

Gender(Gender) SNP_int2_2

Frequency, Percent , Row Pct , Col Pct	Wildtype	Hetero	Homo	Total
Female	2	11	7	20
	3.45	18.97	12.07	34.48
	10.00	55.00	35.00	
	18.18	44.00	31.82	
Male	9	14	15	38
	15.52	24.14	25.86	65.52
	23.68	36.84	39.47	
	81.82	56.00	68.18	
Total	11	25	22	58

show the mean of average INR and Dose by SNP3URT type and SNP_int2_1

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snp_3UTR_type=' Wildtype' SNP_int2_1=' Wildtype'

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	44.8214286	16.8956947	8.4478473
logdose		3.7513871	0.3674594	0.1837297
AVG_INR	AVG_INR	2.2128125	0.5247324	0.2623662

snp_3UTR_type=' Wildtype' SNP_int2_1=Hetero

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	38.5174603	15.2901075	5.4058693
logdose		3.5650191	0.4752387	0.1680222
AVG_INR	AVG_INR	2.6297273	0.5535913	0.1957241

logdose		3.2755736	0.5216568	0.1229557
AVG_INR	AVG_INR	2.5758601	0.4254419	0.1002776

show the mean of average INR and Dose by SNP_3UTR

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---------- SNP_int2_1=' Wildtype' ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	50.1927400	16.3087420	3.1984075
logdose		3.8685025	0.3124001	0.0612667
AVG_INR	AVG_INR	2.3704996	0.4170822	0.0817966

---------- SNP_int2_1=Hetero ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	35.1921249	15.3860425	3.7316634
logdose		3.4646447	0.4633535	0.1123797
AVG_INR	AVG_INR	2.6694068	0.4469707	0.1084063

---------- SNP_int2_1=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	31.1386855	24.0234425	6.2028262
logdose		3.2628553	0.5734644	0.1480679
AVG_INR	AVG_INR	2.6093968	0.4165374	0.1075495

The FREQ Procedure

SNP_int2_1	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Wildtype	26	44.83	26	44.83
Hetero	17	29.31	43	74.14
Homo	15	25.86	58	100.0

show the mean of average INR and Dose by gender and SNP_int2_1

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---------- Gender=Female SNP_int2_1=' Wildtype' ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	55.1053060	20.3946591	6.4493575
logdose		3.9507823	0.3604104	0.1139718
AVG_INR	AVG_INR	2.4474811	0.4816052	0.1522969

---------- Gender=Female SNP_int2_1=Hetero ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	46.9749975	15.8416921	6.4673437
logdose		3.7910179	0.3960118	0.1616712
AVG_INR	AVG_INR	2.5502275	0.3842718	0.1568783

---------- Gender=Female SNP_int2_1=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	51.3415919	40.5043721	20.2521860
logdose		3.7477622	0.6714319	0.3357160
AVG_INR	AVG_INR	2.6926633	0.5852637	0.2926318

---------- Gender=Male SNP_int2_1=' Wildtype' ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	47.1223862	12.9453502	3.2363375
logdose		3.8170776	0.2781813	0.0695453
AVG_INR	AVG_INR	2.3223862	0.3799252	0.0949813

show the catagory of snp_intron2_1

show the mean of average INR and Dose by gender and SNP_int2_1

23:46 Sunday, July 18, 2004

---------- Gender=Male SNP_int2_1=Hetero ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	28.7651035	11.1601955	3.3649256
logdose		3.2866229	0.4078346	0.1229667
AVG_INR	AVG_INR	2.7344137	0.4823375	0.1454302

---------- Gender=Male SNP_int2_1=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	23.7921741	9.6521260	2.9102255
logdose		3.0865256	0.4437392	0.1337924
AVG_INR	AVG_INR	2.5791181	0.3692735	0.1113401

show the mean of average INR and Dose by SNP_int2_2

23:46 Sunday, July 18, 2004

---------- SNP_int2_2='Wildtype' ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	25.2927392	10.1090598	3.0479962
logdose		3.1442028	0.4606249	0.1388836
AVG_INR	AVG_INR	2.6769791	0.4114026	0.1240426

---------- SNP_int2_2=Hetero ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	41.6810176	24.5845179	4.9169036
logdose		3.5838540	0.5454437	0.1090887
AVG_INR	AVG_INR	2.6254942	0.4307616	0.0861523

The FREQ Procedure

Table of SNP_int2_1 by SNP_int2_2

SNP_int2_1	SNP_int2_2			
Frequency	Wildtype	Hetero	Homo	Total
Wildtype	0	4	22	26
Hetero	0	17	0	17
Homo	11	4	0	15
Total	11	25	22	58

The FREQ Procedure

Table of SNP_int2_1 by SNP_3UTR

SNP_int2_1	SNP_3UTR			
Frequency	Wildtype	Hetero	Homo	Total
Wildtype	4	11	11	26
Hetero	8	8	1	17
Homo	15	0	0	15
Total	27	19	12	58

The FREQ Procedure

Table of SNP_int2_2 by SNP_3UTR

SNP_int2_2	SNP_3UTR			
Frequency	Wildtype	Hetero	Homo	Total
Wildtype	11	0	0	11
Hetero	12	12	1	25
Homo	4	7	11	22

---------- snp_3UTR_type=' Wildtype' SNP_int2_1=Homo ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	31.1386855	24.0234425	6.2028262
logdose		3.2628553	0.5734644	0.1480679
AVG_INR	AVG_INR	2.6093968	0.4165374	0.1075495

---------- snp_3UTR_type=mutation SNP_int2_1=' Wildtype' ----------

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	51.1693421	16.4122438	3.4991021
logdose		3.8897962	0.3062777	0.0652986
AVG_INR	AVG_INR	2.3991700	0.4026911	0.0858540

snp_3UTR_type (mutation) and snp_int2_1

50

show the mean of average INR and Dose by SNP3URT type and SNP_int2_1

---------- snp_3UTR_type=mutation SNP_int2_1=Hetero ----------

The MEANS Procedure

Variable	Label	Mean	Std Dev	Std Error
AVG_Dose	AVG_Dose	32.2362712	15.7483200	5.2494400
logdose		3.3754229	0.4612396	0.1537465
AVG_INR	AVG_INR	2.7046775	0.3583800	0.1194600

Table of snp_3UTR_type by SNP_int2_1

snp_3UTR_type \ SNP_int2_1	Wildtype	Hetero	Homo	Total
Wildtype	4	8	15	27
mutation	22	9	0	31
Total	26	17	15	58

Total	27	19	12	58

TAB 34

07/27/2004

Single nucleotide polymorphisms of vitamin K epoxide reductase gene affects S-warfarin dose

Abstract

Patients require different warfarin dose to achieve therapeutic anticoagulation, which might be explained by genetic variability in the vitamin K epoxide reductase (VKOR) gene—the target enzyme of warfarin. Analysis of the coding region, untranslated region and intron region of *VKOR* was undertaken in 58 American patients whose unbound oral clearance of S-warfarin had been previously determined. Five single nucleotide polymorphisms (SNPs) were identified, three of which were found to be related to the patients' warfarin average dose. Female patients had significantly ($P < .^{**}$) greater warfarin dose than male patients. In conclusion, the warfarin sensitivities among patients are determined by multiple SNPs in VKOR gene and Cytochome P450 2C9, which can be used to build a model for the prediction for warfarin dose before therapy.

Introduction

Oral anticoagulants therapy is used to prevent the recurrent arterial and venous thrombosis. Warfarin was first used in 1950s as an anticoagulant for victims of heart attacks and strokes and is the most prescribed anticoagulant in the world. However, the

response to warfarin is influenced by multiple factors such as gender, diet, diabetes, race (citations) and other unknown factors, which makes it hard for the physicians to control the warfarin dose in clinic. Since the subsequent warfarin elimination is due to its oxidation by the Cytochrome P450 2C9, researchers took their efforts on the relationship between the variation of P450 2C9 and warfarin sensitivity and found that P450 2C9*2 and 2C9*3 SNPs affected warfarin sensitivity (citations).

In spite of the widely usage of warfarin, its mechanism as the anticoagulant was not clear until recently two groups identified the vitamin K epoxide reductase (VKOR) gene and published their data in the same issue of Nature (citations). Warfarin targets VKOR and prevents the reuse of vitamin K, which acts as a cofactor of gamma-glutamyl carboxylase, thus decreases the carboxylation of the vitamin K-dependent coagulation proteins and causes bleeding. So we surmise the SNPs in VKOR gene might affect the warfarin sensitivity.

VKOR gene is about 4 kb long, consisting of three exons (the most prevalent isoform). The coding region of VKOR gene is with 492 bp length and the protein is 163 amino acids with a mass of 18.4 KDa.

Methods

1. Patients

2. Extract genomic DNA from whole blood

Genomic DNAs were extracted from the whole blood using QIAamp DNA Blood Mini Kit (QIAGEN cat#51104). Adjust the DNA concentration to 10 ng/μL.

3. Sequence the genomic DNA samples

~10ng DNA was used for PCR reactions. The primers used for amplify the VKOR genes were: Exon1-5' CCAATCGCCGAGTCAGAGG & Exon1-3' CCCAGTCCCCAGCACTGTCT were used to amplify the 5'-UTR and Exon1 region; Exon2-5' AGGGGAGGATAGGGTCAGTG & Exon2-3' CCTGTTAGTTACCTCCCCACA were used to amplify the Exon2 region; Exon3-5' ATACGTGCGTAAGCCACCAC & Exon3-3' ACCCAGATATGCCCCCTTAG were used to amplify the Exon3 and 3'-UTR region. Automated high throughput capillary electrophoresis DNA sequencing was used for detecting SNPs in VKOR gene.

4. Detect the known SNPs using real-time PCR

The assay reagents for SNP genotyping was ordered from the Assay-by-Design™ service (Applied Biosystems, cat#4332072). The primers and probes (FAM™ and VIC™ dye-labeled) were designed using Primer Express software and were synthesized in Applied Biosystems. 2X TaqMan™ Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems, cat#4324018) was used in the PCR reactions. The real-time PCR reactions were performed in Opticon II (MJ Research). 95°C 10 min. preheat, 92°C 15 sec, 60°C 1 min. followed by a plate reading, 40 cycles. The results were read according to signal value of FAM and VIC dye.

5. Statistics for the data

SNP variables were categorized accordingly to the SNPs. The difference of average dose between different groups of genotype was compared by analysis of variance (ANOVA). The examination of the distribution and residuals for the average dose of treatment among the SNP groups indicated that a log transformation was necessary to satisfy the assumption of homogeneity of variance. Importantly, gender has been identified as a significant confounding factor for the average dose of the treatment. Female patients had significantly higher average dose of the treatment ($p=0.013$ by one way ANOVA). After controlling of this confounding factor, the analysis of variance indicated that differences existed between the mean log (average dose) of wild type and of homozygotes. Patients with Homo SNP significantly need more dose of warfarin ($p=0.026$). A table of mean (average dose) and the mean log (average dose) is presented. All statistical analyses were

performed using SAS version 8.0 (SAS, Inc., Cary,NC). A two-sided p value less than 0.05 was considered significant.

6. Predict the mRNA stability using *** program

7. Measure the mRNA stability in vitro

Result and discussion

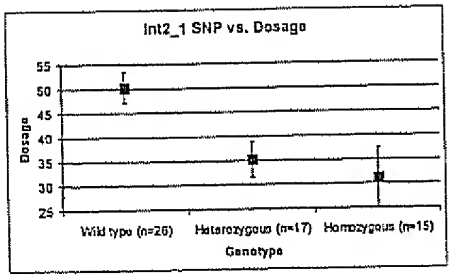
1. The effects of the SNPs on warfarin dosage

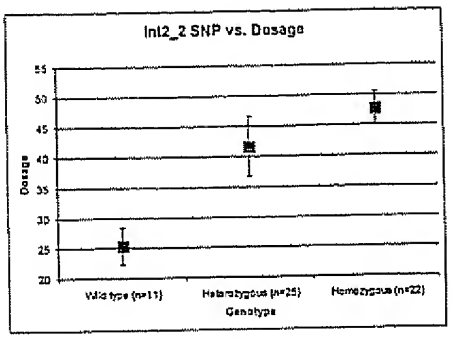
By direct genomic DNA sequencing and SNP real-time PCR detection, we found 5 SNPs in VKOR gene: one in 5'-UTR, two in intron region, one in coding region and one in 3'-UTR (Table 1).

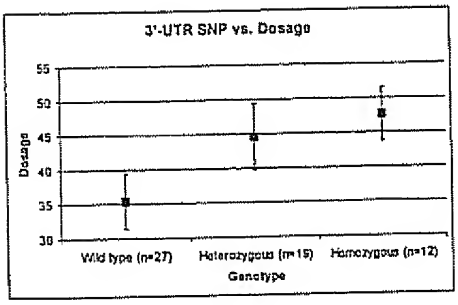
SNPs	NT change	position	AA change	Heterozygous retio
5'-UTR	563G>A	5'-UTR	N/A	1/58
Int2_1	2581G>C	Intron2	N/A	17/58
Int2_2	3294T>C	Intron2	N/A	25/58
Exon3	4501C>T	Exon3	Leu120Leu	1/58
3'-UTR	4769G>A	3'-UTR	N/A	19/58

Among these SNPs, 5'UTR and Exon3 SNPs have only one sample among 58 patients, while the others have 17-25 heterozygous patients.

We did warfarin dosage analysis for the Int2_1, Int2_2 and 3'-UTR SNPs. Figure 1A shows the average dose for patients with Int2_1 SNP wild type is 50.19+/-3.20 (n=26), while the heterozygous and homozygous are 35.19+/-3.73 (n=17) and 31.14+/-6.2 (n=15). The difference between wild type and heterozygous&homozygous are significant (p=***, p=***, respectively). However, Int2_2 and 3'-UTR SNPs seem to have adverse effects on warfarin dosage comparing to Int2_1 SNP. Figure 1B shows the average dose for patients with Int2_2 SNP wild type is 25.29+/-3.05 (n=11), while the heterozygous and homozygous are 41.68+/-4.92 (n=25) and 47.73+/-2.75 (n=22). The difference between wild type and heterozygous&homozygous are significant (p=***, p=***, respectively). Figure 1C shows the average dose for patients with 3'-UTR SNP wild type is 35.35+/-4.01 (n=27), while the heterozygous and homozygous are 44.48+/-4.80 (n=19) and 47.56+/-3.86 (n=12). The difference between wild type and heterozygous&homozygous are significant (p=***, p=***, respectively).







2. Analyze the haplotypes

(Need a genetics specialist to write this part)

SNP_Int2_1 vs. SNP_Int2_2

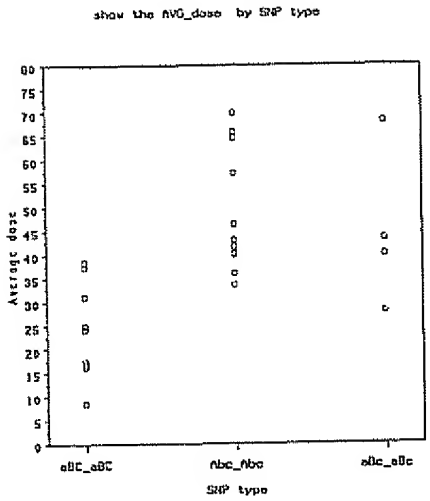
		SNP_Int2_2			
		wildtype	hetero	homo	Total
SNP_Int2_1	wildtype	0	4	22	26
	hetero	0	17	0	17
	homo	11	4	0	15
	Total	11	25	22	58

SNP_Int2_1 vs. SNP_3'UTR

		SNP_3' UTR			
		wildtype	hetero	homo	Total
SNP_Int2_1	wildtype	4	11	11	26
	hetero	8	8	1	17
	homo	15	0	0	15
	Total	27	19	12	58

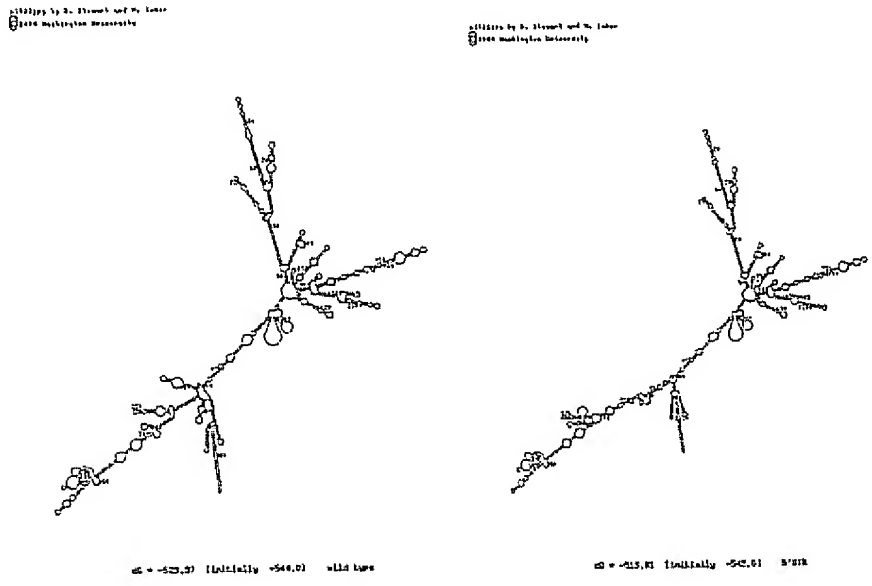
SNP_Int2_2 vs. SNP_3' UTR

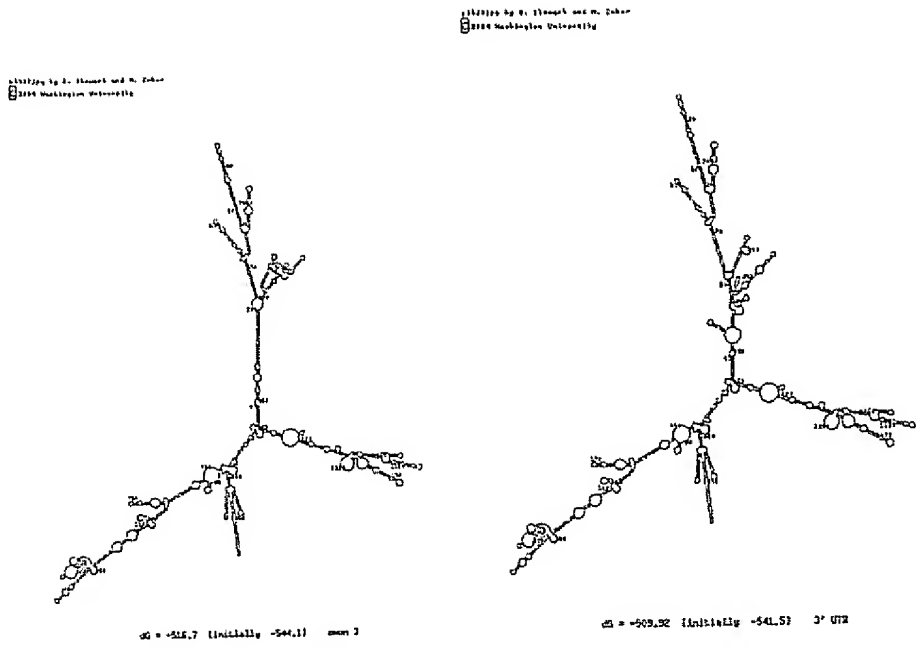
		SNP_3' UTR			
		wildtype	hetero	homo	Total
SNP_Int2_2	wildtype	11	0	0	11
	hetero	12	12	1	25
	homo	4	7	11	22
	Total	27	19	12	58



3. The prediction of the mRNA stabilities

The prediction of mRNA stabilities for the 5'-UTR, Exon3 and 3'-UTR was performed using *** program. The 5'-UTR SNP doesn't seem to affect the mRNA structure much, however, the Exon3 SNP (C/T) and 3'-UTR SNP influence dramatically local stem-loop structures. ACCC- motif in 3'UTR might also influence its binding to a RNA-binding protein.

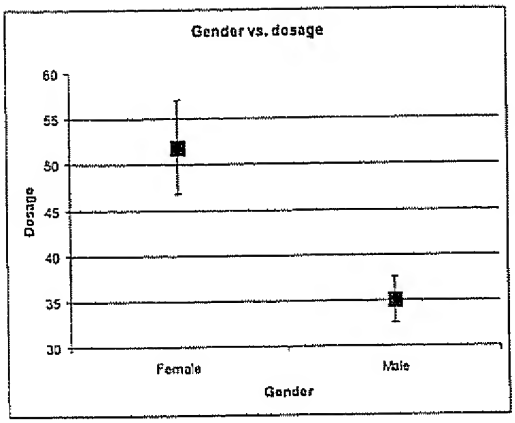


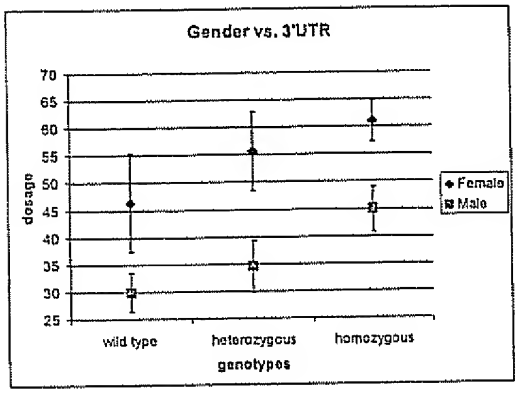


4. Warfarin dosage is different between female and male

During this study, we happened to find that there were apparent warfarin dosage differences between men and women. Among 58 patients, the average warfarin dose for women is 51.91+/-5.17 (n=?) and for men is 35.06+/-2.51 (n=?). The P value for the difference between men and women is ***.

We divided the 58 patient into female and male groups and analyze the influence of 3'-UTR SNP separately. Figure shows that no matter it's wild type, heterozygous or homozygous, the warfarin dose for female is always higher than male, and the trends for both are wild type<heterozygous<homozygous.


Gender vs. dosage
60
55
50
45
40
35
30
Dosage
Female
Male
Gender


Gender vs. 3'UTR
70
65
60
55
50
45
40
35
30
25
dosage
wild type
heterozygous
homozygous
genotypes
Female
Male